US008105812B2

(12) **United States Patent**
Wang et al.

(10) **Patent No.: US 8,105,812 B2**
(45) **Date of Patent: Jan. 31, 2012**

(54) **LACCASES, COMPOSITIONS AND METHODS OF USE**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 794 days.

(21) Appl. No.: **11/954,804**

(22) Filed: **Dec. 12, 2007**

(65) **Prior Publication Data**

US 2008/0196173 A1 Aug. 21, 2008

Related U.S. Application Data

(60) Provisional application No. 60/875,518, filed on Dec. 18, 2006, provisional application No. 60/875,454, filed on Dec. 18, 2006.

(51) **Int. Cl.**
C12N 9/02 (2006.01)

(52) **U.S. Cl.** **435/189**; 536/23.2

(58) **Field of Classification Search** 435/189; 536/23.2

See application file for complete search history.

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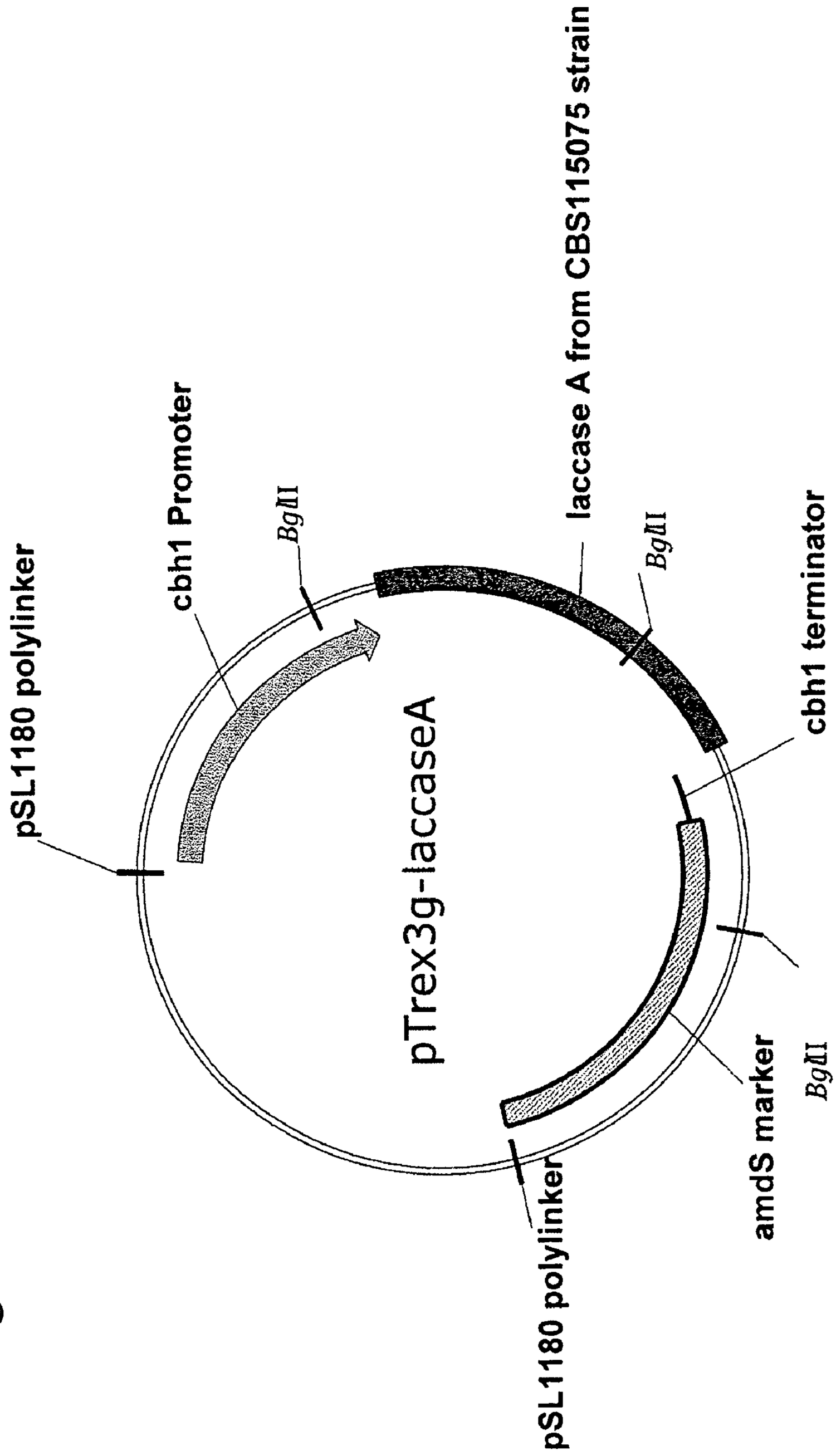
(57) **ABSTRACT**

Novel laccases, nucleic acid sequences encoding such laccases, and vectors and host cells for expressing the laccases are described. The novel laccase enzymes may be employed in conjunction with mediators to provide an improved method for bleaching denim fabrics.

2 Claims, 10 Drawing Sheets


pTrex3g-laccaseA
cbh1 Promoter
BglII
laccase A from CBS115075 strain
BglII
cbh1 terminator
amdS marker
BglII
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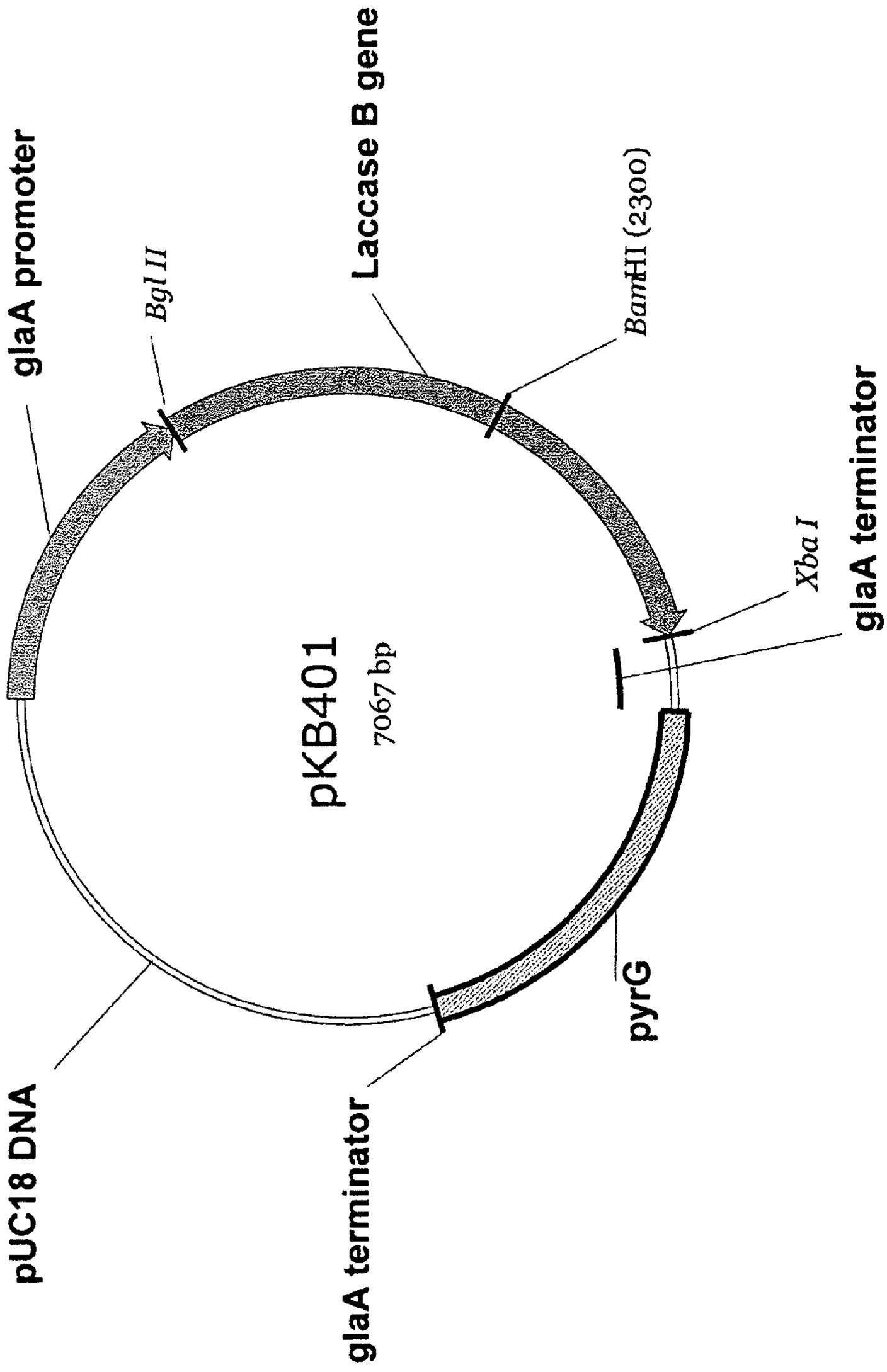
pSL1180 polylinker

Figure 1


glaA promoter
Bgl II
Laccase B gene
BamHI (2300)
Xba I
glaA terminator
pyrG
glaA terminator
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pKB401
7067 bp

Figure 2

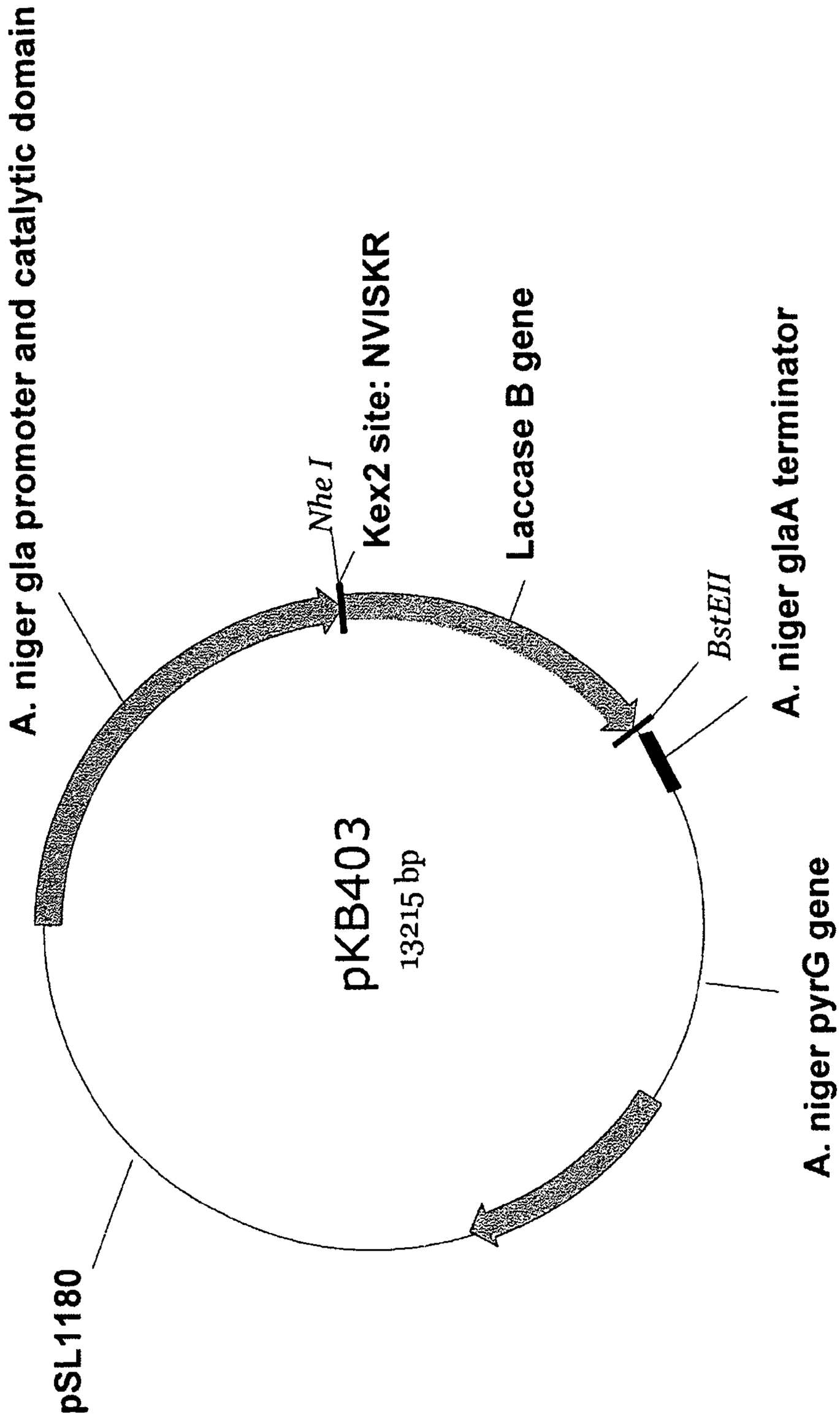

A. niger gla promoter and catalytic domain
Nhe I
Kex2 site: NVISKR
Laccase B gene
BstEII
A. niger glaA terminator
A. niger pyrG gene
pKB403
13215 bp
pSL1180

Figure 3

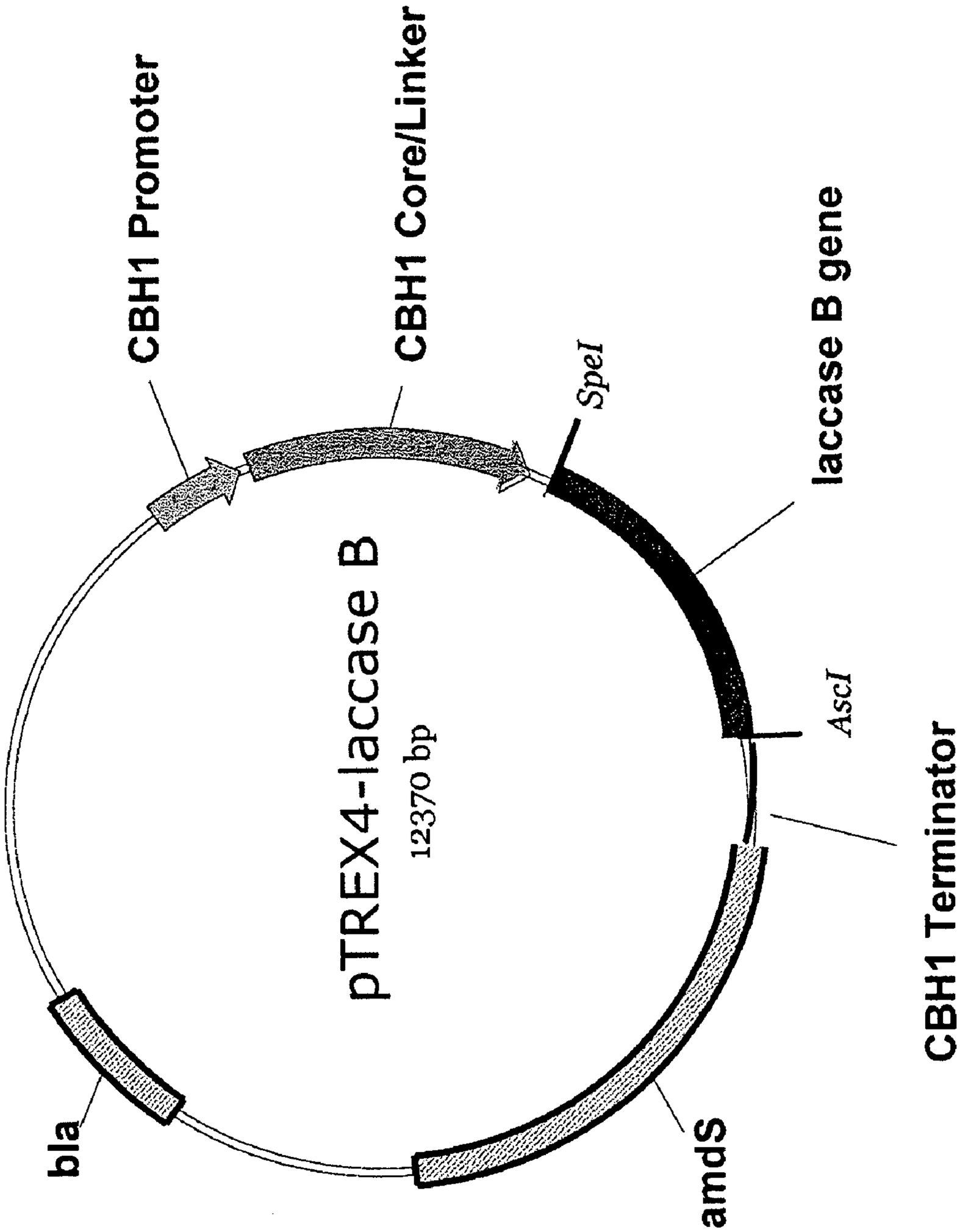
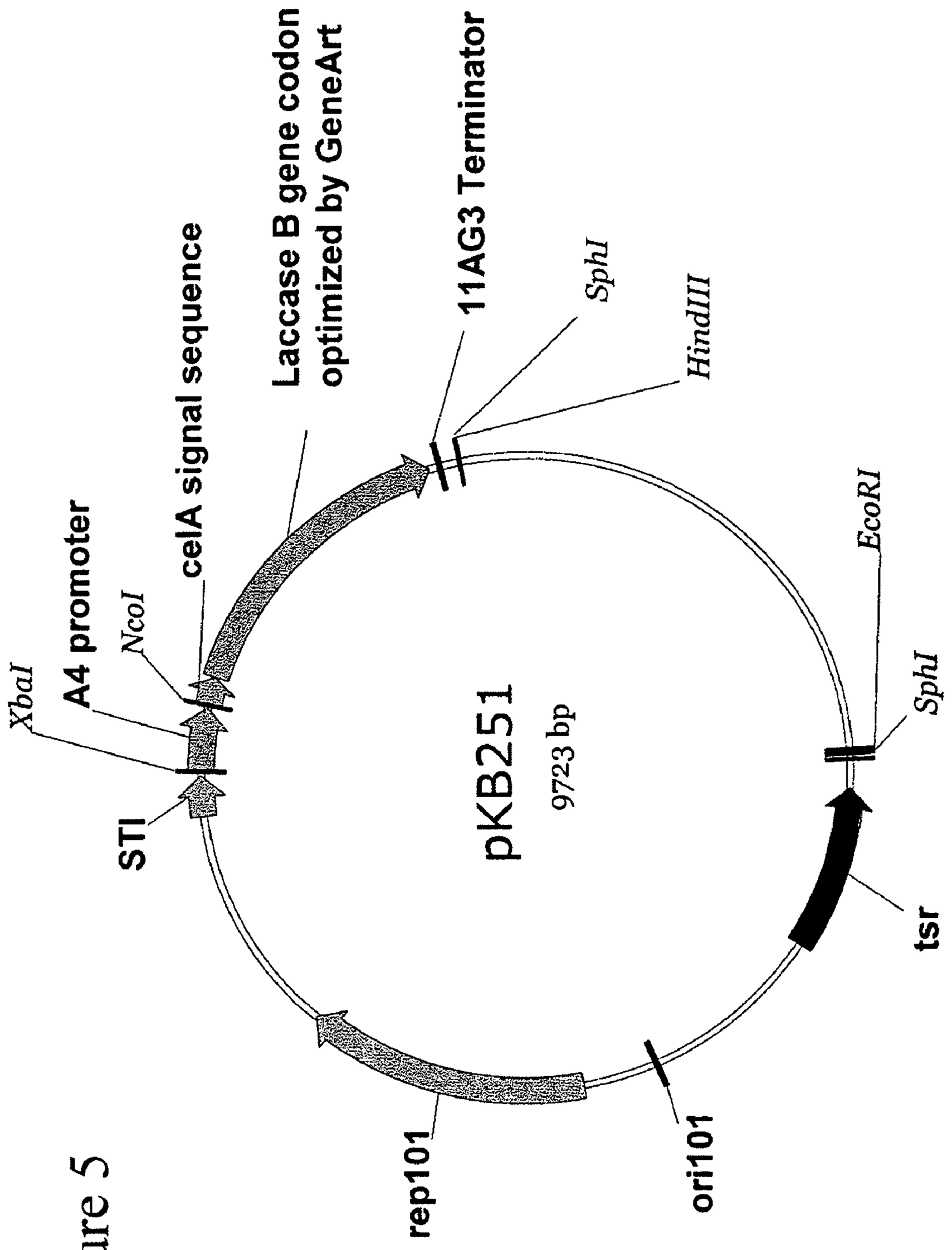

CBH1 Promoter
CBH1 Core/Linker
SpeI
laccase B gene
pTREX4-laccase B
12370 bp
AscI
CBH1 Terminator
bla
amdS

Figure 4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9723 bp
A4 promoter
celA signal sequence
Lac
opti
11AG3 Terminator
STI
rep101
ori101
tsr
XbaI
NcoI
SphI
HindIII
EcoRI
SphI

Figure 5

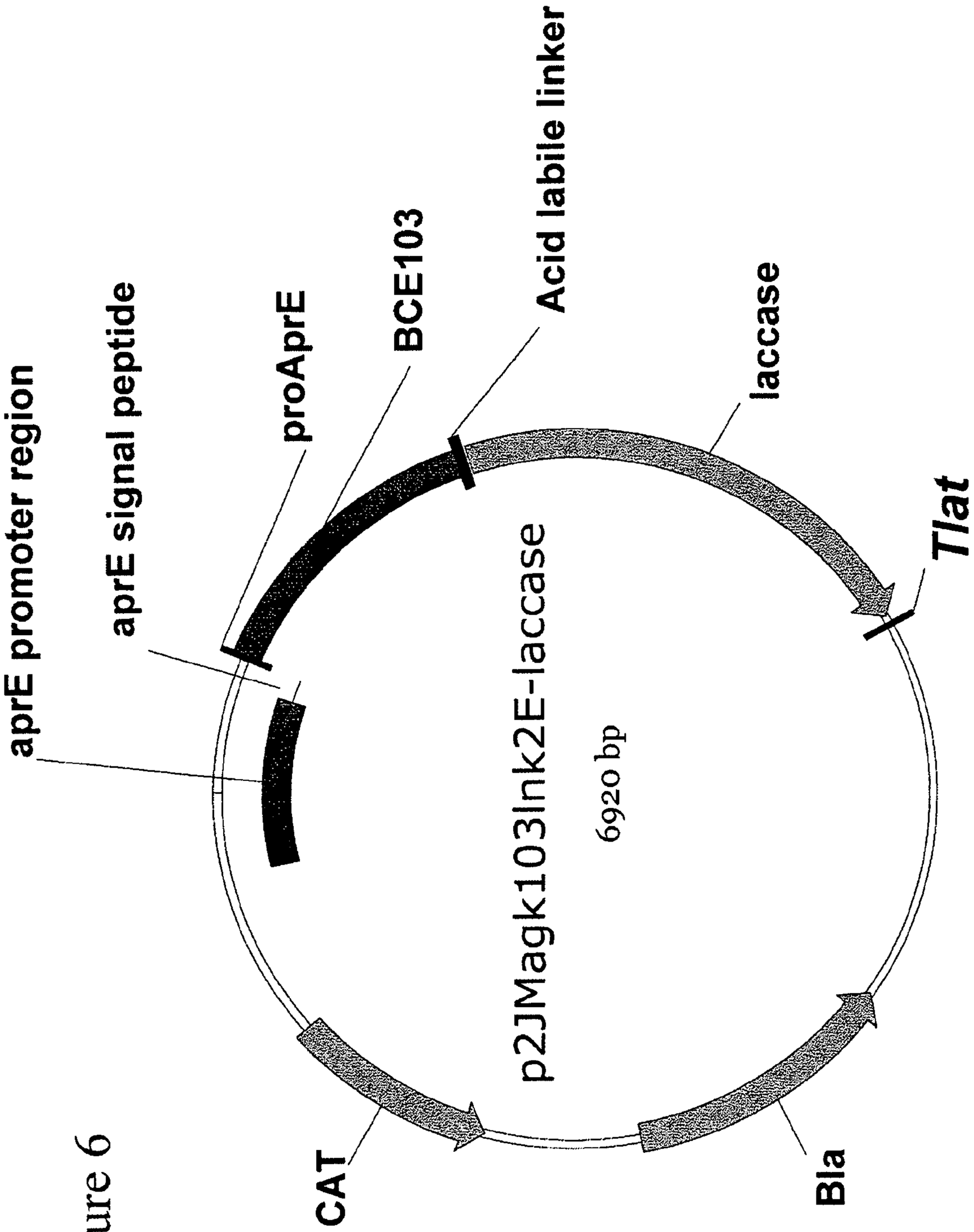

aprE promoter region
aprE signal peptide
proAprE
BCE1
Aci
laccase
Tlat
p2JMagk103lnk2E-laccase
6920 bp
CAT
Bla

Figure 6

Figure 7. Bleaching of soluble indigo using a *Thielavia* sp. laccase and a variety of mediators at 50 and 500 uM concentrations.

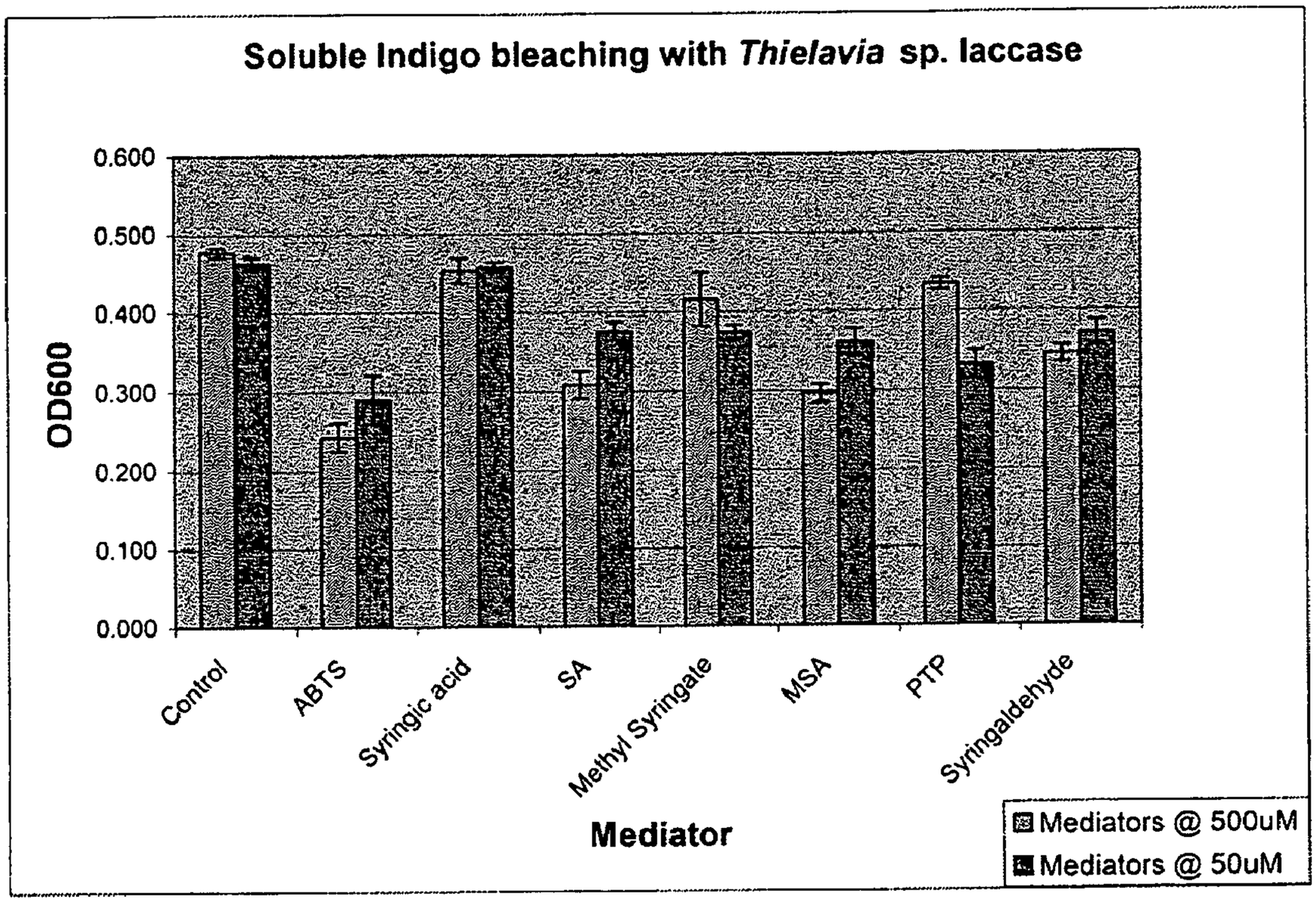


Figure 8. Bleaching of soluble indigo using a *Thielavia*, *Myceliophthora* and *Cerrena* sp. laccase and a variety of mediators at pH 5.

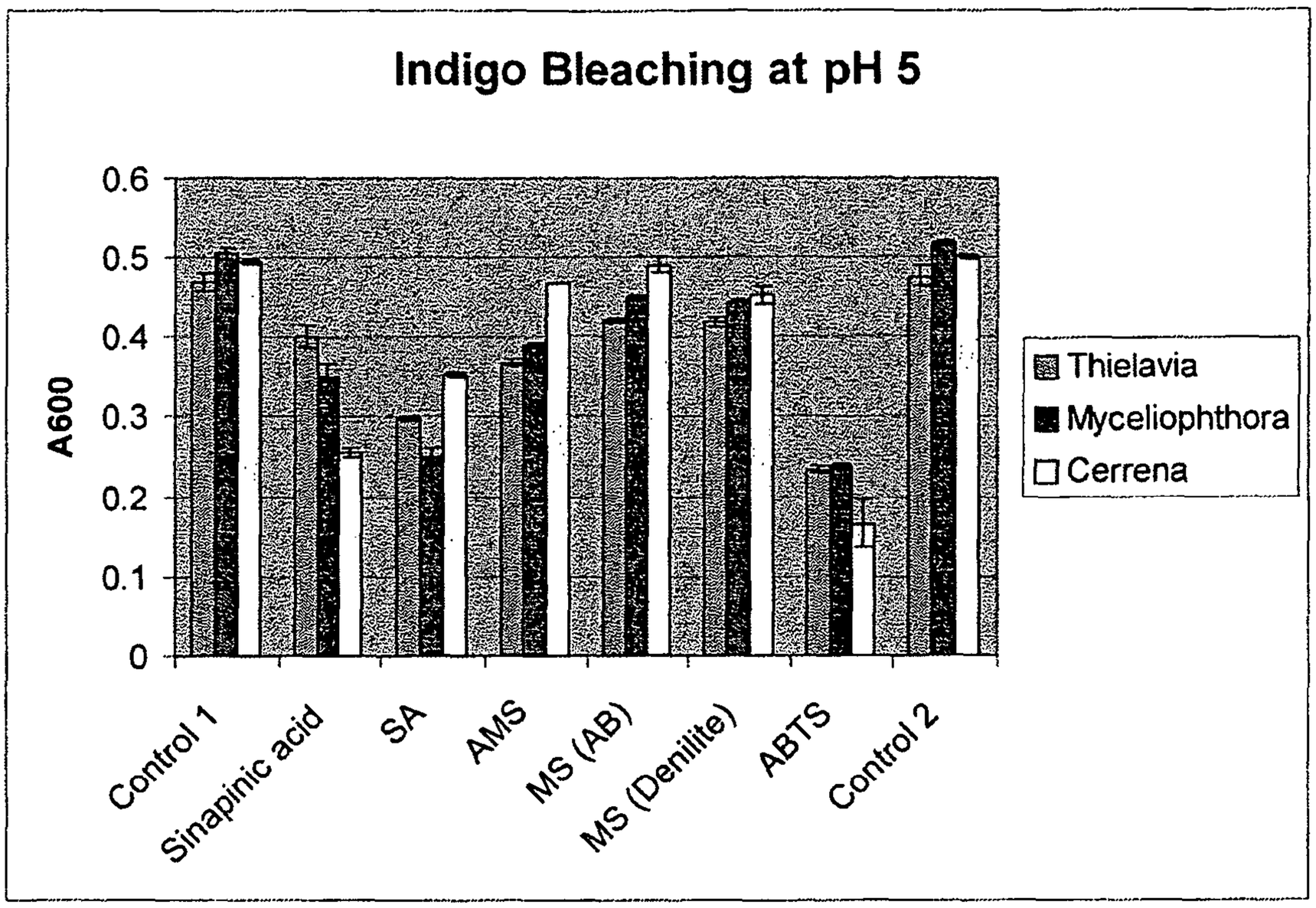
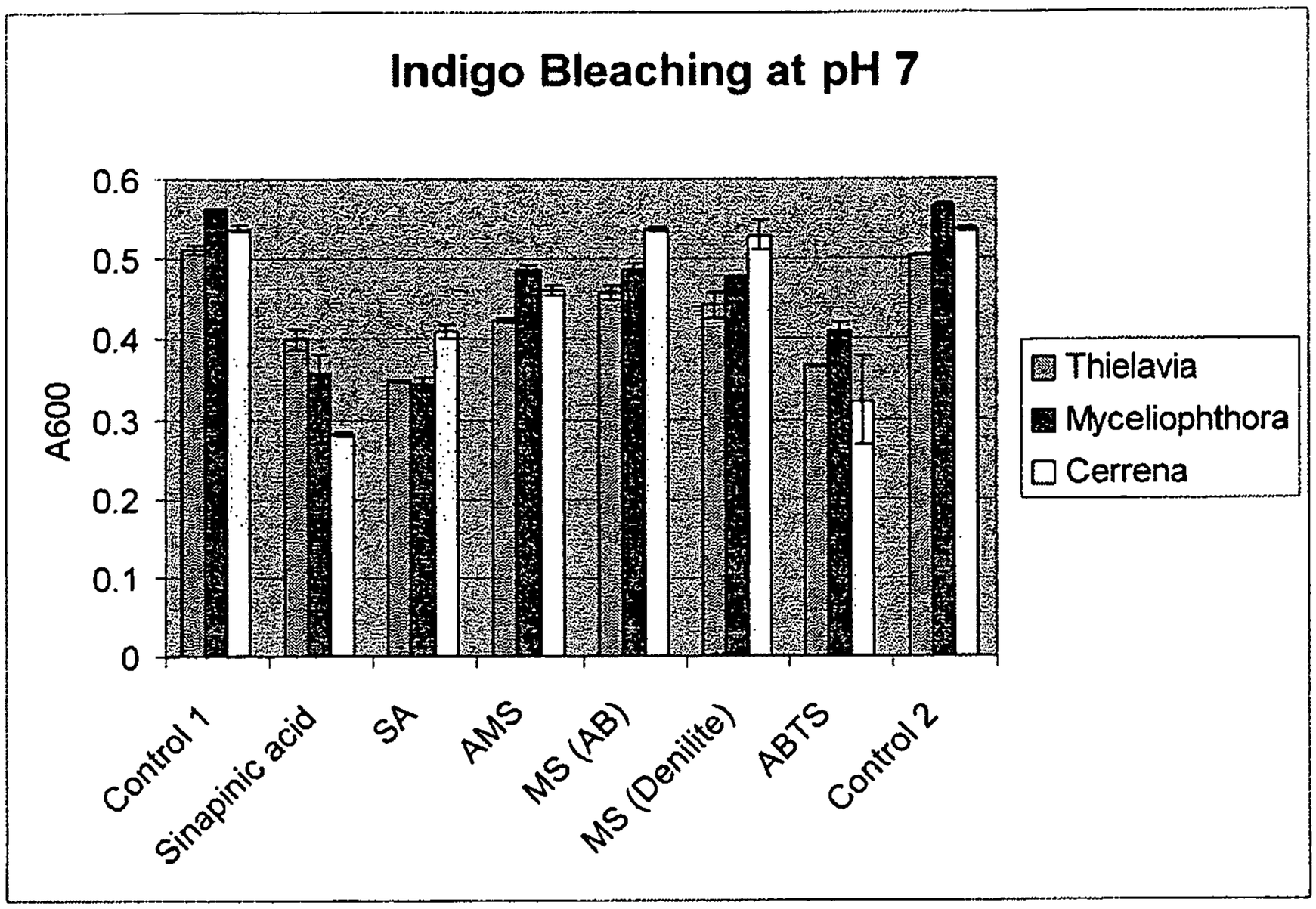


Figure 9. Bleaching of soluble indigo using a *Thielavia*, *Myceliophthora* and *Cerrena* sp. laccase and a variety of mediators at pH 7.



GC942 Figures (for Examples 7-12)

Figure 10

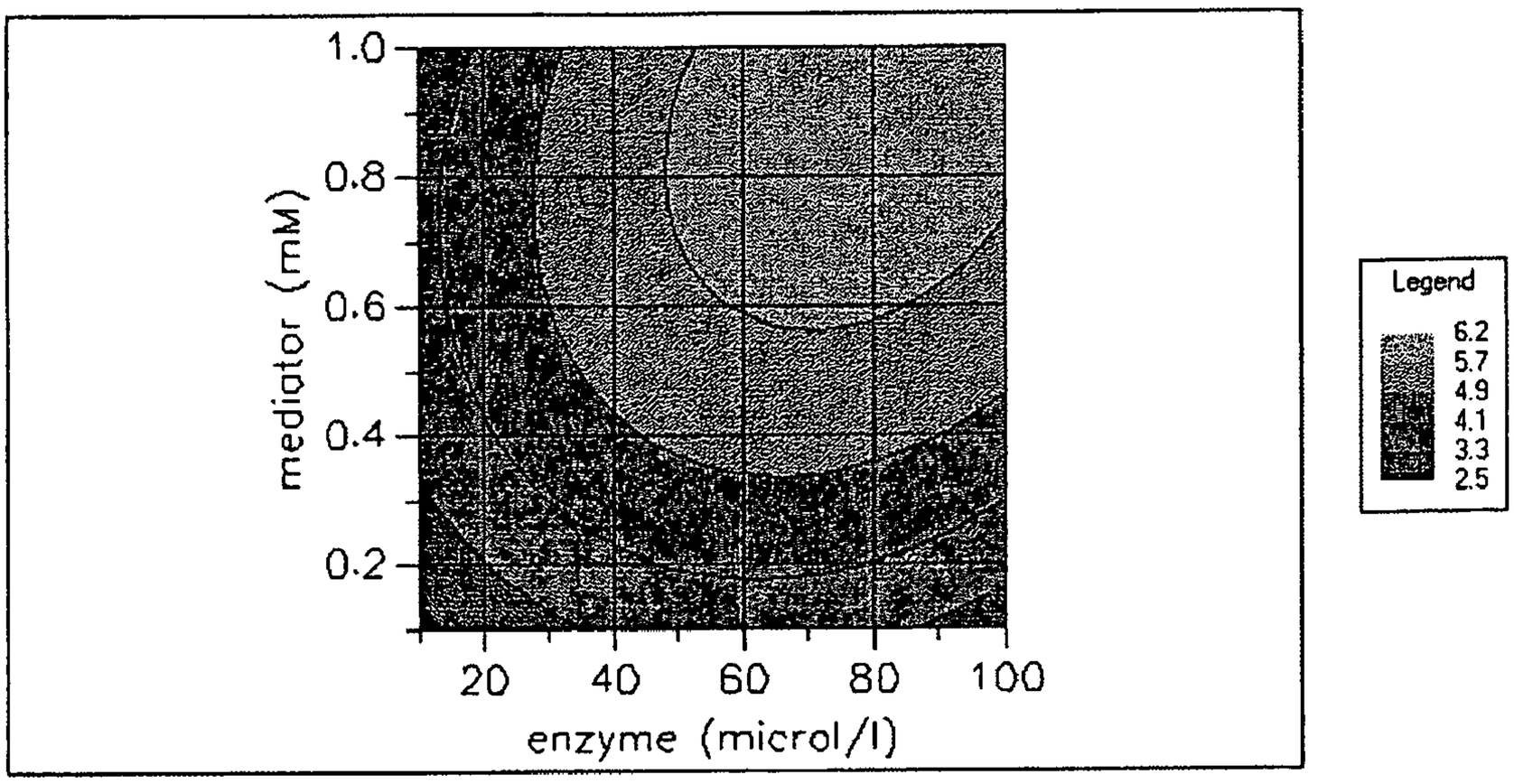
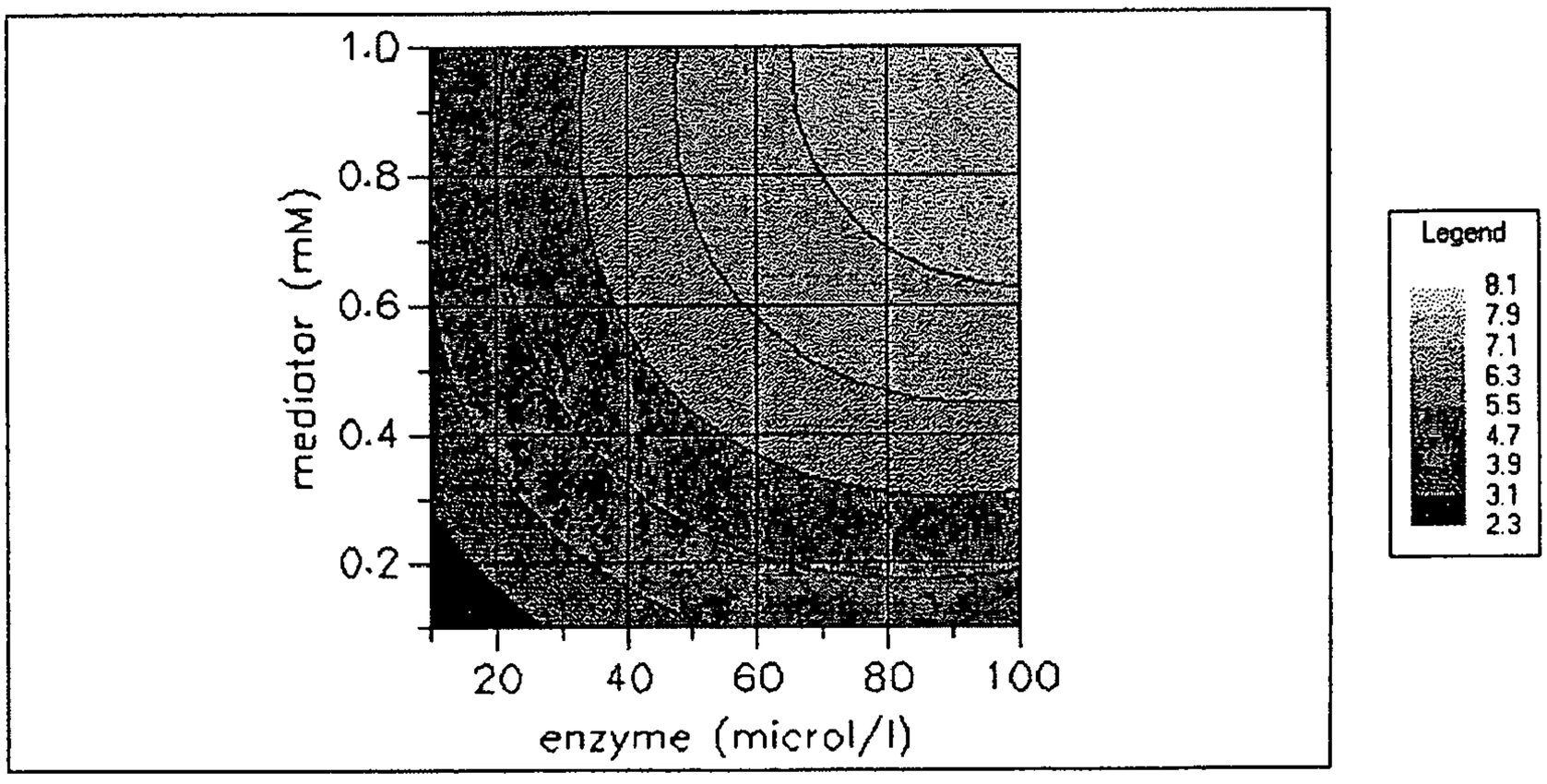


Figure 11



LACCASES, COMPOSITIONS AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority to U.S. Provisional Patent Application Ser. No. 60/875,518, entitled "Novel Laccases, Compositions and Methods of Use", filed 18 Dec. 2006 and U.S. Provisional Patent Application Ser. No. 60/875,454, entitled "Laccase Mediators and Methods of Use", filed 18 Dec. 2006.

FIELD OF THE INVENTION

The present invention relates to laccases and nucleic acid sequences encoding the laccases, and to enzymatic methods for bleaching materials.

BACKGROUND OF THE INVENTION

Laccases are copper-containing enzymes that are known to be good oxidizing agents in the presence of oxygen. Laccases are found in microbes, fungi, and higher organisms. Laccase enzymes are used for many applications, including pulp and textiles bleaching, treatment of pulp waste water, de-inking, industrial color removal, bleaching laundry detergents, oral care teeth whiteners, and as catalysts or facilitators for polymerization and oxidation reactions.

Laccases can be utilized for a wide variety of applications in a number of industries, including the detergent industry, the paper and pulp industry, the textile industry and the food industry. In one application, phenol oxidizing enzymes are used as an aid in the removal of stains, such as food stains, from clothes during detergent washing.

Most laccases exhibit pH optima in the acidic pH range while being inactive in neutral or alkaline pHs.

Laccases are known to be produced by a wide variety of fungi, including species of the genii *Aspergillus, Neurospora, Podospora, Botrytis, Pleurotus, Fornes, Phlebia, Trametes, Polyporus, Stachybotrys, Rhizoctonia, Bipolaris, Curvularia, Amerosporium*, and *Lentinus*. However, there remains a need for laccases having different performance profiles in various applications.

For many applications, the oxidizing efficiency of a laccase can be improved through the use of a mediator, also known as an enhancing agent. Systems that include a laccase and a mediator are known in the art as laccase-mediator systems (LMS). The same compounds can also be used to activate or initiate the action of laccase.

There are several known mediators for use in a laccase-mediator system. These include HBT (1-hydroxybenzotriazole), ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfinic acid)], NHA (N-hydroxyacetanilide), NEIAA (N-acetyl-N-phenylhydroxylamine), HBTO (3-hydroxy 1,2,3-benzotriazin-4(3H)-one), and VIO (violuric acid). In addition, there are several compounds containing NH—OH or N—O that have been found to be useful as mediators.

Functional groups and substituents have large effects on mediator efficiency. Even within the same class of compounds, a substituent can change the laccase specificity towards a substrate, thereby increasing or decreasing mediator efficiency greatly. In addition, a mediator may be effective for one particular application but unsuitable for another application. Another drawback for current mediators is their tendency to polymerize during use. Thus, there is a need to discover efficient mediators for specific applications. One

such application is the bleaching of textiles, wherein it is also important that the mediators are not unduly expensive or hazardous. Other applications of the laccase-mediator system are given below.

Thus, there is a need to identify additional mediators that activate laccase, and/or enhance the activity of enzymes that exhibit laccase activity.

SUMMARY OF THE INVENTION

Described herein are novel laccases, nucleic acid sequences encoding such laccases, and vectors and host cells for expressing the laccases.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** is a schematic of the *Trichoderma* expression plasmid, pTrex3g-laccaseA, used in Example 7. The laccase A gene may be replaced with other laccase genes described herein.

FIG. **2** is a schematic of the *Aspergillus* expression plasmid, pKB401, used in Example 8a. The laccase B gene may be replaced with other laccase genes described herein.

FIG. **3** is a schematic of the *Aspergillus* expression plasmid, pKB403, used in Example 8b. The laccase B gene fused to gene encoding the catalytic domain of glucoamylase. The laccase B gene may be replaced with other laccase genes described herein.

FIG. **4** is a schematic of the *Trichoderma* expression plasmid, pTrex4-laccaseB, used in Example 8d. The laccase B gene fused to gene encoding the catalytic domain of CBH1. The laccase B gene may be replaced with other laccase genes described herein.

FIG. **5** is a schematic of the *Streptomyces* expression plasmid (pKB251) for codon optimized laccase B gene, used in Example 9.

FIG. **6** is a schematic of the *Bacillus* expression plasmid (p2JMagk103lnk2E-laccase) for codon optimized laccase D gene fused to the gene encoding BCE103, used in Example 13.

FIG. **7** is a bar graph showing the results of bleaching soluble indigo using a *Thielavia* sp. laccase and a variety of mediators at 50 and 500 uM concentrations.

FIG. **8** is a bar graph showing the results of bleaching of soluble indigo using a *Thielavia, Myceliophthora* and *Cerrena* sp. laccase and a variety of mediators at pH 5.

FIG. **9** is a bar graph showing the results of bleaching of soluble indigo using a *Thielavia, Myceliophthora* and *Cerrena* sp. laccase and a variety of mediators at pH 7.

FIG. **10** is a total color difference graph for the recombinant laccase D and syringamide mediator as a function of mediator concentration and enzyme concentration at 60° C. and pH 6.

FIG. **11** is a total color difference graph for the recombinant laccase D and syringonitrile mediator as a function of mediator concentration and enzyme concentration at 60° C. and pH 6.

DETAILED DESCRIPTION OF THE INVENTION

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton, et al., DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY, 2D ED., John Wiley and Sons, New York (1994), and Hale & Marham, THE HARPER COLLINS DICTIONARY OF BIOLOGY, Harper Perennial, N.Y. (1991) provide one of

skill with a general dictionary of many of the terms used in this invention. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. Numeric ranges are inclusive of the numbers defining the range. It is to be understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these may vary.

The headings provided herein are not limitations of the various aspects or embodiments of the invention which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification as a whole.

All publications cited herein are expressly incorporated herein by reference for the purpose of describing and disclosing compositions and methodologies which might be used in connection with the invention.

I. Laccase and Laccase Related Enzymes

In the context of this invention, laccases and laccase related enzymes contemplate any laccase enzyme comprised by the enzyme classification (EC 1.10.3.2). The laccase enzymes are known from microbial and plant origin. The microbial laccase enzyme may be derived from bacteria or fungi (including filamentous fungi and yeasts) and suitable examples include a laccase derivable from a strain of *Aspergillus, Neurospora*, e.g. *N. crassa. Podospora, Botrytis, Collybia, Cerrena, Stachybotrys, Panus*,e.g., *Panus rudis, Theilava, Fomes, Lentinus, Pleurotus, Trametes*, e.g. *T. villosa* and *T. versicolor, Rhizoctonia*, e.g. *R. solani, Coprinus*, e.g. *C. plicatilis* and *C. cinereus, Psatyrella, Myceliophthora*, e.g. *M. thermonhila, Schytalidium, Phlebia*, e.g., *P. radita* (WO 92/01046), or *Coriolus*, e.g. *C. hirsutus* (JP 2--238885), *Spongipellis* sp., *Polyporus, Ceriporiopsis subvermispora, Ganoderma tsunodae* and *Trichoderma.*

The laccase or the laccase related enzyme may furthermore be produced by a method comprising cultivating a host cell transformed with a recombinant DNA vector which carries a DNA sequence encoding said laccase as well as DNA sequences permitting the expression of the DNA sequence encoding the laccase, in a culture medium under conditions permitting the expression of the laccase enzyme, and recovering the laccase from the culture.

The expression vector may be transformed into a suitable host cell, such as a fungal cell, preferred examples of which are species of *Aspergillus*, most preferably *Aspergillus oryzae* and *Aspergillus niger*, and species of *Fusarium*, most preferably *Fusarium venenatum*. Fungal cells may be transformed by a process involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a manner known per se. The use of *Aspergillus* as a host microorganism is described in EP 238,023. The use of *Fusarium* as a host microorganism is described in WO 96/00787 and WO 97/08325.

Alternatively, the host organism may be a bacterium, in particular strains of *Bacillus, Pseudomonas, Streptomyces*, or *E. coli*. The transformation of bacterial cells may be performed according to conventional methods, e.g., as described in T. Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, 1982. The screening of appropriate DNA sequences and construction of vectors may also be carried out by standard procedures, cf. T. Maniatis et al., op. cit.

The medium used to cultivate the transformed host cells may be any conventional medium suitable for growing the host cells in question. The expressed enzyme may conveniently be secreted into the culture medium and may be recovered therefrom by well-known procedures including separating the cells from the medium by centrifugation or filtration, precipitating proteinaceous components of the medium by means of a salt such as ammonium sulphate, followed by chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

In an embodiment, the expression host may be a *Trichoderma reesei* with the laccase coding region under the control of a CBH1 promoter and terminator. (See, e.g., U.S. Pat. No. 5,861,271). The expression vector may be pTrex3g, as disclosed in U.S. patent application Ser. No. 11/245,628 filed 7 Oct. 2005.

In this manner the following novel genes and laccases were prepared:

A. *Cerrena* laccase A1 gene from CBS115.075 strain (SEQ ID No. 1) having the sequence

```
ATGAGCTCAA AGCTACTTGC TCTTATCACT GTCGCTCTCG TCTTGCCACT    50
AGGCACCGAC GCCGGCATCG GTCCTGTTAC CGACTTGCGC ATCACCAACC   100
AGGATATCGC TCCAGATGGC TTCACCCGAC CAGCGGTACT AGCTGGGGGC   150
ACATTCCCTG GAGCACTTAT TACCGGTCAG AAGGTATGGG AGATCAACTT   200
GGTTGAATAG AGAAATAAAA GTGACAACAA ATCCTTATAG GGAGACAGCT   250
TCCAAATCAA TGTCATCGAC GAGCTTACCG ATGCCAGCAT GTTGACCCAG   300
ACATCCATTG TGAGTATAAT TTAGGTCCGC TCTTCTGGCT ATCCTTTCTA   350
ACTCTTACCG TCTAGCATTG GCACGGCTTC TTTCAGAAGG GATCTGCGTG   400
GGCCGATGGT CCTGCCTTCG TTACTCAATG CCCTATCGTC ACCGGAAATT   450
CCTTCCTGTA CGACTTTGAT GTTCCCGACC AACCTGGTAC TTTCTGGTAC   500
CATAGTCACT TGTCTACTCA ATATTGCGAT GGTCTTCGTG GCCCGTTCGT   550
TGTATACGAT CCAAAGGATC CTAATAAACG GTTGTACGAC ATTGACAATG   600
GTATGTGCAT CATCATAGAG ATATAATTCA TGCAGCTACT GACCGTGACT   650
GATGCTGCCA GATCATACGG TTATTACCCT GGCAGACTGG TACCACGTTC   700
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-continued

TCGCAAGAAC TGTTGTCGGA GTCGCGTAAG TACAGTCTCA CTTATAGTGG 750

TCTTCTTACT CATTTTGACA TAGGACACCC GACGCAACCT TGATCAACGG 800

TTTGGGCCGT TCTCCAGACG GGCCAGCAGA TGCTGAGTTG GCTGTCATCA 850

ACGTTAAACG CGGCAAACGG TATGTTATTG AACTCCCGAT TTCTCCATAC 900

ACAGTGAAAT GACTGTCTGG TCTAGTTATC GATTTCGTCT GGTCTCCATC 950

TCATGTGACC CTAATTACAT CTTTTCTATC GACAACCATT CTATGACTGT 1000

CATCGAAGTC GATGGTGTCA ACACCCAATC CTGACCGTC GATTCTATTC 1050

AAATCTTCGC AGGCCAACGA TACTCGTTCG TCGTAAGTCT CTTTGCACGA 1100

TTACTGCTTC TTTGTCCATT CTCTGACCTG TTTAAACAGC TCCATGCCAA 1150

CCGTCCTGAA AACAACTATT GGATCAGGGC CAAACCTAAT ATCGGTACGG 1200

ATACTACCAC AGACAACGGC ATGAACTCTG CCATTCTGCG ATACAACGGC 1250

GCACCTGTTG CGGAACCGCA AACTGTTCAA TCTCCCAGTC TCACCCCTTT 1300

GCTCGAACAG AACCTTCGCC CTCTCGTGTA CACTCCTGTG GTATGTTTCA 1350

AAGCGTTGTA ATTTGATTGT GGTCATTCTA ACGTTACTGC GTTTGCATAG 1400

CCTGGAAACC CTACGCCTGG CGGCGCCGAT ATTGTCCATA CTCTTGACTT 1450

GAGTTTTGTG CGGAGTCAAC ATTCGTAAAG ATAAGAGTGT TTCTAATTTC 1500

TTCAATAATA GGATGCTGGT CGCTTCAGTA TCAACGGTGC CTCGTTCCTT 1550

GATCCTACCG TCCCCGTTCT CCTGCAAATT CTCAGCGGCA CGCAGAATGC 1600

ACAAGATCTA CTCCCTCCTG GAAGTGTGAT TCCTCTCGAA TTAGGCAAGG 1650

TCGTCGAATT AGTCATACCT GCAGGTGTCG TCGGTGGACC TCATCCGTTC 1700

CATCTCCATG GGGTACGTAA CCCGAACTTA TAACAGTCTT GGACTTACCC 1750

GCTGACAAGT GCATAGCATA ACTTCTGGGT CGTGCGAAGT GCCGGAACCG 1800

ACCAGTACAA CTTTAACGAT GCCATTCTCC GAGACGTCGT CAGTATAGGA 1850

GGAACCGGGG ATCAAGTCAC CATTCGTTTC GTGGTATGTT TCATTCTTGT 1900

GGATGTATGT GCTCTAGGAT ACTAACCGGC TTGCGCGTAT AGACCGATAA 1950

CCCCGGACCG TGGTTCCTCC ATTGCCATAT CGACTGGCAC TTGGAAGCGG 2000

GTCTCGCTAT CGTATTTGCA GAGGGAATTG AAAATACTGC TGCGTCTAAT 2050

TTAACCCCCC GTACGCGGTT TCCCTCACAT CCTGGAGCTA AGCAGCTTAC 2100

TAACATACAT TTGCAGAGGC TTGGGATGAG CTTTGCCCGA AGTATAACGC 2150

GCTCAGCGCA CAAAAGAAGG TTGCATCTAA GAAAGGCACT GCCATCTAAT 2200

TTTTGTAACA AACAAGGAGG GTCTCTTGTA CTTTTATTGG GATTTCTTTC 2250

TTGGGGTTTA TTGTTAAACT TGACTCTACT ATGTTTGGAA GACGAAAGGG 2300

GCTCGCGCAT TTATATACTA TCTCTCTTGG CATCACCTGC AGCTCAATCC 2350

TTCAACCACC TAA 2363

encoding the enzyme laccase A1, having the translated protein sequence (SEQ ID No. 2)

MSSKLLALIT VALVLPLGTD AGIGPVTDLR ITNQDIAPDG FTRPAVLAGG 50

TFPGALITGQ KGDSFQINVI DELTDASMLT QTSIHWHGFF QKGSAWADGP 100

AFVTQCPIVT GNSFLYDFDV PDQPGTFWYH SHLSTQYCDG LRGPFVVYDP 150

KDPNKRLYDI DNDHTVITLA DWYHVLARTV VGVATPDATL INGLGRSPDG 200

PADAELAVIN VKRGKRYRFR LVSISCDPNY IFSIDNHSMT VIEVDGVNTQ 250

SLTVDSIQIF AGQRYSFVLH ANRPENNYWI RAKPNIGTDT TTDSGMNSAI 300

-continued

LRYNGAPVAE PQTVQSPSLT PLLEQNLRPL VYTPVPGNPT PGGADIVHTL 350

DLSFDAGRFS INGASFLDPT VPVLLQILSG TQNAQDLLPP GSVIPLELGK 400

VVELVIPAGV VGGPHPFHLH GHNFWVVRSA GTDQYNFNDA ILRDVVSIGG 450

TGDQVTIRFV TDNPGPWFLH CHIDWHLEAG LAIVFAEGIE NTAASNLTPQ 500

AWDELCPKYN ALSAQKKLNP STT 523

B. *Cerrena* laccase A2 gene from CBS154.29 strain
(SEQ ID No. 3)

ATGAGCTCAA AGCTACTTGC TCTTATTACT GTCGCTCTCG TCTTGCCACT 50

AGGCACTGAC GCCGGCATCG GTCCTGTTAC CGACTTGCGC ATCACCAACC 100

AGGATATCGC TCCAGATGGC TTCACCCGAC CAGCTGTACT GGCTGGGGGC 150

ACATTCCCCG GAGCACTGAT TACCGGTCAG AAGGTATGGG AGATCGATTT 200

CGTTGAATAG AGAAATACAA CTGAAAACAA ATTCTTATAG GGAGACAGCT 250

TCCAAATCAA TGTCATCGAC GAGCTTACCG ATGCCAGCAT GTTGACCCAG 300

ACATCCATTG TGAGTATAAT ATGGGTCCGC TCTTCTAGCT ATCCTTTCTA 350

ACTCTTACCC TCTAGCATTG GCACGGCTTC TTTCAGAAGG GATCTGCGTG 400

GGCCGATGGT CCTGCCTTCG TTACTCAATG TCCTATCGTC ACCGGAAATT 450

CCTTCCTGTA CGACTTTGAT GTCCCCGACC AACCTGGTAC TTTCTGGTAC 500

CATAGTCACT TGTCTACTCA ATATTGCGAT GGTCTTCGGG GCCCGTTCGT 550

TGTATACGAT CCAAAGGATC CTAATAAACG GTTGTACGAC ATTGACAATG 600

GTATGTGCAT CATCATAAAA ATATAATTCA TGCAGCTACT GACCGCGACT 650

GATGCTGCCA GATCATACGG TTATTACCCT GGCAGACTGG TACCACGTTC 700

TCGCACGAAC TGTTGTCGGA GTCGCGTAAG TACAGTCTGA CTTATAGTGG 750

TCTTCTTACT CATTTTGACA TAGGACACCC GACGCAACCT TGATCAACGG 800

TTTGGGCCGT TCTCCAGACG GGCCAGCAGA TGCTGAGTTG GCTGTCATCA 850

ACGTTAAACG CGGCAAACGG TATGTCATTG AACTCCCGAT TTCTCCATTC 900

ACATTGAAAT GACTGTCTGG TCTAGTTATC GATTCCGTCT GGTCTCCATC 950

TCATGTGACC CTAATTACAT CTTTTCTATC GACAACCATT CTATGACTGT 1000

CATCGAAGTC GATGGTGTCA ACACCCAATC CCTGACCGTC GATTCTATCC 1050

AAATCTTCGC AGGCCAACGC TACTCGTTCG TCGTAAGTCT CTTTGAATGG 1100

TTGGTGCTTT TTCTGTCCAT TCTCTAACCT GTTTATACAG CTCCATGCCA 1150

ACCGTCCTGA AAACAACTAT TGGATCAGGG CCAAACCTAA TATCGGTACG 1200

GATACTACCA CAGACAACGG CATGAACTCT GCCATTCTGC GATACAACGG 1250

CGCACCTGTT GCGGAACCGC AAACTGTTCA ATCTCCCAGT CTCACCCCTT 1300

TGCTCGAACA GAACCTTCGC CCTCTCGTGT ACACTCCTGT GGTATGTTTC 1350

AAAGCGTTGT AATTTGATTG TGGTCATTCT AACGTTACTG CCTTTGCACA 1400

GCCTGGAAAT CCTACGCCTG GCGGGGCCGA TATTGTCCAT ACTCTTGACT 1450

TGAGTTTTGT GCGGAGTCAA CATTCGTAAA GATAAGAGTG TTTCTAATTT 1500

CTTCAATAAT AGGATGCTGG TCGCTTCAGT ATCAACGGTG CCTCGTTCCT 1550

TGATCCTACC GTCCCTGTTC TCCTGCAAAT TCTCAGCGGC ACGCAGAATG 1600

CACAAGATCT ACTCCCTCCT GGAAGTGTGA TTCCTCTCGA ATTAGGCAAG 1650

GTCGTCGAAT TAGTCATACC TGCAGGTGTT GTCGGTGGAC CTCATCCGTT 1700

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CCATCTCCAT GGGGTACGTA ACCCGAACTT ATAACAGTCT TGGACTTACC  1750

CGCTGACAAG TGTATAGCAT AACTTCTGGG TCGTGCGAAG TGCCGGAACC  1800

GACCAGTACA ACTTTAACGA TGCCATTCTC CGAGACGTCG TCAGTATAGG  1850

AGGAACCGAG GATCAAGTCA CCATTCGATT CGTGGTATAT ACTTCATTCT  1900

TGTGGATGTA TGTGCTCTAG GATACTAACT GGCTTGCGCG TATAGACCGA  1950

TAACCCCGGA CCGTGGTTCC TCCATTGCCA TATCGACTGG CACTTGGAAG  2000

CGGGTCTCGC TATCGTATTT GCAGAGGGAA TTGAAAATAC TGCTGCGTCT  2050

AATCCAACCC CCCGTATGCG GTTTCCCACA CATTCTGAAT CTAAGCAGCT  2100

TACTAATATA CATTTGCAGA GGCTTGGGAT GAGCTTTGCC CGAAGTATAA  2150

CGCGCTCAAC GCACAAAAGA AGGTTGCATC TAAGAAAGGC ACTGCCATCT  2200

AATCCTTGTA ACAAACAAGG AGGGTCTCTT GTACTTTTAT TGGGATTTAT  2250

TTCTTGGGGT TTATTGTTCA ACTTGATTCT ACTATGTTTG GAAGTAGCGA  2300

TTACGAAAGG GGCTTGCGCA TTTATATACC ATCTTTCTTG GCACCACCTG  2350

CAGCTCAATC CTTCAACCAC CTAA                              2374
```

encoding the enzyme laccase A2, having the translated protein sequence shown in (SEQ ID No. 4)

```
MSSKLLALIT VALVLPLGTD AGIGPVTDLR ITNQDIAPDG FTRPAVLAGG    50

TFPGALITGQ KGDSFQINVI DELTDASMLT QTSIHWHGFF QKGSAWADGP   100

AFVTQCPIVT GNSFLYDFDV PDQPGTFWYH SHLSTQYCDG LRGPFVVYDP   150

KDPNKRLYDI DNDHTVITLA DWYHVLARTV VGVATPDATL INGLGRSPDG   200

PADAELAVIN VKRGKRYRFR LVSISCDPNY IFSIDNHSMT VIEVDGVNTQ   250

SLTVDSIQIF AGQRYSFVLH ANRPENNYWI RAKPNIGTDT TTDNGMNSAI   300

LRYNGAPVAE PQTVQSPSLT PLLEQNLRPL VYTPVPGNPT PGGADIVHTL   350

DLSFDAGRFS INGASFLDPT VPVLLQILSG TQNAQDLLPP GSVIPLELGK   400

VVELVIPAGV VGGPHPFHLH GHNFWVVRSA GTDQYNFNDA ILRDVVSIGG   450

TEDQVTIRFV TDNPGPWFLH CHIDWHLEAG LAIVFAEGIE NTAASNPTPQ   500

AWDELCPKYN ALNAQKKLNP STT                               523
```

C. *Cerrena* laccase B1 gene from CBS115.075 strain (SEQ ID No. 5)

```
ATGTCTCTTC TTCGTAGCTT GACCTCCCTC ATCGTACTAG TCATTGGTGC    50

ATTTGCTGCA ATCGGTCCAG TCACTGACCT ACATATAGTG AACCAGAATC   100

TCGACCCAGA TGGTTTCAAC CGCCCCACTG TACTCGCAGG TGGTACTTTC   150

CCCGGTCCTC TGATTCGTGG TAACAAGGTA CGCTTCATAA CCGCCCTCCG   200

TAGACGTAGG CTTCGGCTGA CATGACCATC ATCTGTAGGG AGATAACTTT   250

AAAATTAATG TGATTGACGA CTTGACAGAG CACAGTATGC TCAAGGCTAC   300

GTCCATCGTA AGTCCCTGAT TAACGTTTCA CCTGGTCATA TCGCTCAACG   350

TCTCGAAGCA CTGGCATGGG TTCTTCCAGA AGGGAACCAA CTGGGCCGAT   400

GGCCCCGCCT TTGTCACCCA ATGTCCTATC ACATCAGGAA ACGCCTTCCT   450

GTATGATTTC AACGTTCCGG ACCAAGCTGG TACTTTCTGG TACCACAGCC   500

ATCTCTCTAC ACAGTATTGT GACGGTCTTC GTGGTGCCTT TGTCGTCTAT   550

GATCCTAATG ATCCCAACAA GCAACTCTAT GATGTTGATA ACGGCAAGTT   600

CCTTGCATAT TTCATTTCTA TCATATCCTC ACCTGTATTG GCACAGAAAG   650

CACCGTGATT ACCTTGGCTG ATTGGTATCA TGCCCTTGCT CAGACTGTCA   700
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CTGGTGTCGC GTGAGTGACA AATGGCCCTC AATTGTTCAC ATATTTTCCT   750

GATTATCATA TGATAGAGTA TCTGATGCAA CGTTGATCAA CGGATTGGGA   800

CGTTCGGCCA CCGGCCCCGC AAATGCCCCT CTGGCGGTCA TCAGTGTCGA   850

GCGGAATAAG AGGTCAGTTC CATAATTATG ATTATTTCCC GCGTTACTTC   900

CTAACAATTA TTTTTGTATC CCTCCACAGA TATCGTTTCC GATTGGTTTC   950

TATTTCTTGC GACCCTAACT TTATTTTCTC AATTGACCAC CACCCAATGA  1000

CCGTAATTGA GATGGACGGT GTTAATACCC AATCTATGAC CGTAGATTCG  1050

ATCCAAATAT TCGCAGGTCA ACGATATTCA TTTGTCGTAG GTTATTATAA  1100

ACTGCCCACC GATCATCTCT CACGTAACTG TTATAGATGC AAGCCAACCA  1150

ACCAGTTGGA AATTATTGGA TCCGCGCTAA ACCTAATGTT GGGAACACAA  1200

CTTTCCTTGG AGGCCTGAAC TCCGCTATAT TACGATATGT GGGAGCCCCT  1250

GACCAAGAAC CGACCACTGA CCAAACACCC AACTCTACAC CGCTCGTTGA  1300

GGCGAACCTA CGACCCCTCG TCTATACTCC TGTGGTATGT TGTTCTCGTT  1350

ACATATACCA AACCTAATAT GAAGACTGAA CGGATCTACT AGCCGGGACA  1400

GCCATTCCCT GGCGGTGCTG ATATCGTCAA GAACTTAGCT TTGGGTTTCG  1450

TACGTGTATT TCACTTCCCT TTTGGCAGTA ACTGAGGTGG AATGTATATA  1500

GAATGCCGGG CGTTTCACAA TCAATGGAGC GTCCCTCACA CCTCCTACAG  1550

TCCCTGTACT ACTCCAGATC CTCAGTGGTA CTCACAATGC ACAGGATCTT  1600

CTCCCAGCAG GAAGCGTGAT CGAACTTGAA CAGAATAAAG TTGTCGAAAT  1650

CGTTTTGCCC GCTGCGGGCG CCGTTGGCGG TCCTCATCCT TTTCACTTAC  1700

ATGGTGTAAG TATCAGACGT CCTCATGCCC ATATTGCTCC GAACCTTACA  1750

CACCTGATTT CAGCACAATT TCTGGGTGGT TCGTAGCGCC GGTCAAACCA  1800

CATACAATTT CAATGATGCT CCTATCCGTG ATGTTGTCAG TATTGGCGGT  1850

GCAAACGATC AAGTCACGAT CCGATTTGTG GTATGTATCT CGTGCCTTGC  1900

ATTCATTCCA CGAGTAATGA TCCTTACACT TCGGGTTCTC AGACCGATAA  1950

CCCTGGCCCA TGGTTCCTTC ACTGTCACAT TGACTGGCAT TTGGAGGCTG  2000

GGTTCGCTGT AGTCTTTGCG GAGGGAATCA ATGGTACTGC AGCTGCTAAT  2050

CCAGTCCCAG GTAAGACTCT CGCTGCTTTG CGTAATATCT ATGAATTTAA  2100

ATCATATCAA TTTGCAGCGG CTTGGAATCA ATTGTGCCCA TTGTATGATG  2150

CCTTGAGCCC AGGTGATACA TGA                               2173
```

encoding the enzyme laccase B1, having the translated protein sequence (SEQ ID No. 6)

```
MSLLRSLTSL IVLVIGAFAA IGPVTDLHIV NQNLDPDGFN RPTVLAGGTF    50

PGPLIRGNKG DNFKINVIDD LTEHSMLKAT SIHWHGFFQK GTNWADGPAF   100

VTQCPITSGN AFLYDFNVPD QAGTFWYHSH LSTQYCDGLR GAFVVYDPND   150

PNKQLYDVDN GNTVITLADW YHALAQTVTG VAVSDATLIN GLGRSATGPA   200

NAPLAVISVE RNKRYRFRLV SISCDPNFIF SIDHHPMTVI EMDGVNTQSM   250

TVDSIQIFAG QRYSFVMQAN QPVGNYWIRA KPNVGNTTFL GGLNSAILRY   300

VGAPDQEPTT DQTPNSTPLV EANLRPLVYT PVPGQPFPGG ADIVKNLALG   350

FNAGRETING ASLTPPTVPV LLQILSGTHN AQDLLPAGSV IELEQNKVVE   400

IVLPAAGAVG GPHPFHLHGH NFWVVRSAGQ TTYNENDAPI RDVVSIGGAN   450
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DQVTIRFVTD NPGPWFLHCH IDWHLEAGFA VVFAEGINGT AAANPVPAAW 500

NQLCPLYDAL SPGDT 515

D. *Cerrena* laccase B2 gene from CBS154.29 strain
(SEQ ID No. 7)

CACCGCGATG TCTCTTCTTC GTAGCTTGAC CTCCCTCATC GTACTAGCCA 50

CTGGTGCATT TGCTGCAATC GGTCCAGTCA CCGACCTACA TATAGTGAAC 100

CAGAATCTCG CCCCAGATGG TTTAAACCGC CCCACTGTAC TCGCAGGTGG 150

TACTTTCCCC GGTCCTCTGA TTCGTGGTAA CAAGGTACGC TTCATAACCG 200

CCCTCCGTAG ACGTAGGCTT CGGCTGACAT GACCATCATC TGTAGGGAGA 250

TAACTTTAAA ATTAATGTGA TTGACGACTT GACAGAACAC AGTATGCTCA 300

AGGCTACGTC CATTGTAAGT CCCTGATTAA CGTTTCACCT GGTCATATCG 350

CTCAACGTCT CGAAGCACTG GCATGGGTTC TTCCAGAAGG GAACCAACTG 400

GGCCGATGGC CCCGCCTTTG TCACCCAATG TCCTATCACA TCAGGAAACG 450

CCTTCTTGTA TGATTTCAAC GTTCCGGACC AAGCTGGTAC TTTCTGGTAC 500

CACAGCCATC TCTCYACACA GTATTGTGAC GGTCTTCGTG GTGCCTTTGT 550

CGTCTATGAT CCTAATGATC CCAACAAGCA ACTCTATGAT GTTGATAACG 600

GCAAGTCCCT TGCATATTTC AGTTCTATCA TATCCTCACC TGTATTGGCA 650

CAGAAAGCAC CGTGATTACC TTGGCTGATT GGTATCATGC CCTTGCTCAG 700

ACTGTCACTG GTGTCGCGTG AGTGACAAAT GGCCCTTAAT TGTTCACATA 750

TTTTCCTGAT TATCATATGA TAGAGTATCT GATGCAACGT TGATCAACGG 800

ATTGGGACGT TCGGCCACCG GCCCCGCAAA TGCCCCTCTG GCGGTCATCA 850

GTGTCGAGCG GAATAAGAGG TCAGTTCCAT AATTATGATT ATTTCCCGCG 900

TTACTTCCTA ACGATTATTT TTGTATCCCT CCACAGATAT CGTTTCCGAT 950

TGGTTTCTAT TTCTTGCGAC CCTAACTTTA TTTTCTCAAT TGACCACCAC 1000

CCAATGACCG TAATTGAGAT GGACGGTGTT AATACCCAAT CTATGACCGT 1050

AGATTCGATC CAAATATTCG CAGGTCAACG ATATTCATTT GTCGTAGGTT 1100

ATTATAAACT GCCCACCGAT CATCTCTCAC GTAACTGTTA TAGATGCAAG 1150

CCAACCAACC AGTTGGAAAT TATTGGATCC GYGCTAAACC TAATGTTGGG 1200

AACACAACTT TCCTTGGAGG CCTGAACTCC GCTATATTAC GATATGTGGG 1250

AGCCCCTGAC CAAGAACCGA CCACTGACCA AACACCCAAC TCTACACCGC 1300

TCGTCGAGGC GAACCTACGT CCCCTCGTCT ATACTCCTGT GGTATGTTGT 1350

TCTCGTTACA TATACCAAAC CTAATATGAG GACTGAACGG ATCTACTAGC 1400

CGGGACAGCC ATTCCCTGGC GGTGCTGATA TCGTCAAGAA CTTAGCTTTG 1450

GGTTTCGTAC GTGTATTTCA CTTCCCTTTT GGCAGTAACT GAGGTGGAAT 1500

GTATATAGAA TGCCGGGCGT TTCACAATCA ATGGAACATC CTTCACACCT 1550

CCTACAGTCC CTGTACTACT CCAGATCCTC AGTGGTACTC ACAATGCACA 1600

GGATCTTCTT CCAGCAGGAA GCGTGATCGA ACTTGAACAG AATAAAGTTG 1650

TCGAAATCGT TCTGCCCGCT GCGGGCGCCG TTGGCGGTCC TCATCCTTTC 1700

CACTTACATG GTGTAAGTAT CAGACGTCCT CATGCCTATA TTGCTCCGAA 1750

CCTTACACAC CTGATTTCAG CACAATTTCT GGGTGGTTCG TAGCGCCGGT 1800

CAAACCACAT ACAATTTCAA TGATGCTCCT ATCCGTGATG TTGTCAGTAT 1850

TGGCGGTGCA AACGATCAAG TCACGATCCG ATTTGTGGTA TGTATCTCGT 1900

-continued

GCCTTGCATT CATTCCACGA GTAATGATCC TTACACTTCG GGTTCTCAGA 1950

CCGATAACCC TGGCCCATGG TTCCTTCACT GTCACATTGA CTGGCATTTG 2000

GAGGCTGGGT TCGCTGTAGT CTTTGCGGAG GGAATCAATG GCACTGCAGC 2050

TGCTAATCCA GTCCCAGGTA AGACTCTCGC TGCTTTGCGT AATATCTATG 2100

AATTTAAAGC ATATCAATTT GCAGCGGCTT GGAATCAATT GTGCCCGTTG 2150

TATGATGCCT TGAGCCCAGG tGATACATGA TTACTCGTAG CTGTGCTTTC 2200

TTATACATAT TCTATGGGTA TATCGGAGTA GCTGTACTAT AGTATGTACT 2250

ATACTAGGTG GGATATGYTG ATGTTGATTT ATATAATTTT GTTTGAAGAG 2300

TGACTTTATC GACTTGGGAT TTAGCCGAGT ACATACTGAT CTCTCACTAC 2350

AGGCTTGTTT TGTCTTTGGG CGCTTACTCA ACAGTTGACT GTTTTTGCTA 2400

TTACGCATTG AACCGCATTC CGGTCYGACT CGTGTCCTCT ACTGTGACTT 2450

GTATTGGCAT TCTAGCACAT ATGTCTCTTA CCTATAGGAA CAATATGTCT 2500

CAACACTGTT CCAAAACCTG CGTAAACCAA ATATCGTCCA TCAGATCAGA 2550

TCATTAACAG TGCCGCACTA ACCTAATACA CTGGCARGGA CTGTGGAAAT 2600

CCCTATAAAT GACCTCTAGA CCGTGAGGTC ATTGCAAGGT CGCTCTCCTT 2650

GTCAAGATGA CCC 2663

encoding the enzyme laccase B2, having the translated protein sequence (SEQ ID No. 8)

MSLLRSLTSL IVLATGAFAA IGPVTDLHIV NQNLAPDGLN RPTVLAGGTF 50

PGPLIRGNKG DNFKINVIDD LTEHSMLKAT SIHWHGFFQK GTNWADGPAF 100

VTQCPITSGN AFLYDFNVPD QAGTFWYHSH LSTQYCDGLR GAFVVYDPND 150

PNKQLYDVDN GNTVITLADW YHALAQTVTG VAVSDATLIN GLGRSATGPA 200

NAPLAVISVE RNKRYRFRLV SISCDPNFIF SIDHHPMTVI EMDGVNTQSM 250

TVDSIQIFAG QRYSFVMQAN QPVGNYWIRA KPNVGNTTFL GGLNSAILRY 300

VGAPDQEPTT DQTPNSTPLV EANLRPLVYT PVPGQPFPGG ADIVKNLALG 350

FNAGRETING TSFTPPTVPV LLQILSGTHN AQDLLPAGSV IELEQNKVVE 400

IVLPAAGAVG GPHPFHLHGH NFWVVRSAGQ TTYNFNDAPI RDVVSIGGAN 450

DQVTIRFVTD NPGPWFLHCH IDWHLEAGFA VVFAEGINGT AAANPVPAAW 500

NQLCPLYDAL SPGDT 515

E. *Cerrena* laccase B3 gene (partial) from ATCC20013 strain (SEQ ID No. 9)

GTGGGGGCGG ATCCCTAACT GTTTCGAATC GGCACCGAAG TATGCAGGTG 50

TGACGGAGAT GAGGCGTTTT TTCATCTTCC ACTGCAGTAT AAAATGTCTC 100

AGGTAACGTC CAGCTTTTTG TACCAGAGCT ACCTCCAAAT ACCTTTACTC 150

GCAAAGGTTT CGCGATGTCT CTTCTTCGTA GCTTGACCTC CCTCATCGTA 200

CTAGCCACTG GTGCATTTGC TGCAATCGGT CCAGTCACTG ACCTACATAT 250

AGTGAACCAG AATCTCGCCC CAGATGGTTT CAACCGCCCC ACTGTACTCG 300

CAGGTGGTAC TTTCCCCGGT CCTCTGATTC GTGGTAACAA GGTACGCTTC 350

ATAACCGCCC TCCGTAGACG TAGGCTTCGG CTGACATGAC CATCATCTGT 400

AGGGAGATAA CTTTAAAATT AATGTGATTG ACGACTTGAC AGAACACAGT 450

ATGCTCAAGG CCACGTCCAT TGTAAGTCCC TGATTAACGT TTCACCTGGT 500

CATATCGCTC AACGTCTCGA AGCACTGGCA TGGGTTCTTC CAGAAGGGAA 550

-continued

CCAACTGGGC CGATGGCCCC GCCTTTGTCA CCCAATGTCC TATCACATCA 600

GGAAACTCCT TCCTGTATGA TTTCAACGTT CCGGACCAAG CTGGTACTTT 650

CTGGTACCAC AGCCATCTCT CTACACAGTA TTGTGACGGT CTTCGTGGTG 700

CCTTTGTCGT CTATGATCCT AATGATCCCA ACAAGCAACT CTATGATGTT 750

GATAACGGCA AGTCCCTTGC ATATTTCATT TCTATCATAT CCTCACCTGT 800

ATTGGCACAG AAAGCACCGT GATTACCTTG GCTGATTGGT ATCATGCCCT 850

TGCTCAGACT GTCACTGGTG TCGCGTGAGT GACAAATGGC CCTCAATTGT 900

TCACATATTT TCCTGATTAT CATATGATAG AGTATCTGAT GCAACGTTGA 950

TCAACGGATT GGGACGTTCG GCCACCGGCC CCGCAAATGC CCCTCTGGCG 1000

GTCATCAGTG TCGAGCGGAA TAAGAGGTCA GTTCCATAAT TATGATTATT 1050

TCCCGCGTTA CTTCCTAACA ATTATTCTTG TATCCCTCCA CAGATATCGC 1100

TTCCGATTGG TGTCTATTTC TTGCGACCCT AACTTTATTT TCTCAATTGA 1150

TCACCACCCA ATGACCGTAA TTGAGATGGA CGGTGTTAAT ACCCAATCTA 1200

TGACCGTAGA TTCGATCCAA ATATTCGCAG GTCAACGATA TTCATTTGTC 1250

GTAGGTTATT ATAAACTGCC CACCGATCAT CTCTCACGTA ACTGTTATAG 1300

ATGCAAGCCA ACCAACCRGT TGGAAATTAT TGGATCC 1337

encoding the enzyme laccase B3, having the partial translated protein sequence (SEQ ID No. 10)

MSLLRSLTSL IVLATGAFAA IGPVTDLHIV NQNLAPDGFN RPTVLAGGTF 50

PGPLIRGNKG DNFKINVIDD LTEHSMLKAT SIHWHGFFQK GTNWADGPAF 100

VTQCPITSGN SFLYDFNVPD QAGTFWYHSH LSTQYCDGLR GAFVVYDPND 150

PNKQLYDVDN GKTVITLADW YHALAQTVTG VAVSDATLIN GLGRSATGPA 200

NAPLAVISVE RNKRYRFRLV SISCDPNFIF SIDHHPMTVI EMDGVNTQSM 250

TVDSIQIFAG QRYSFVMQAN QPVGNYWI 278

F. *Cerrena* laccase C gene (partial) from CBS154.29 strain (SEQ ID No. 11)

TGCAATCGGA CCGGTBGCTG ACCTTCACAT TACGGACGAT ACCATTGCCC 50

CCGATGGTTT CTCTCGTCCT GCTGTTCTCG CTGGCGGGGG TTTCCCTGGC 100

CCTCTCATCA CCGGAAACAA GGTAATGCCT AATGGTTGCG TCTTTGTTGG 150

TGCTCTCATT CATCCACGAC ATTTTGTACC AGGGCGACGC CTTTAAACTC 200

AATGTCATCG ATGAACTAAC GGACGCATCC ATGCTGAAGY CGACTTCCAT 250

CGTAAGTCTC GCTGTATTGC TCCTTGAGCC ATTTCATTGA CTATAACTAC 300

AACCAGCACT GGCATGGATT CTTCCAAAAG GGTACTAATT GGGCAGATGG 350

TCCCGCTTTT GTGAACCAAT GCCCCATCAC CACGGGAAAC TCCTTCTTGT 400

ACGACTTCCA GGTTCCTGAT CAAGCTGGTA AGCATGAGAT TACACTAGGA 450

AAGTTTAATT TAATAACTAT TCAATCAGGA ACCTACTGGT ATCATAGTCA 500

TTTGTCTACG CAATACTGTG ATGGTCTCAG AGGTGCATTC GTTGTCTACG 550

ACCCTTCAGA TCCTCACAAG GATCTCTACG ACGTCGACGA CGGTGAGCTT 600

TGCTTTTTTC ATTGGTATCC ATTATCGCTC ACGTGTCATT ACTGCGCCAC 650

AGAAAGTACC GTCATCACTT TGGCTGATTG GTATCATACT TTGGCTCGTC 700

AGATTGTTGG CGTTGCGTGA GTAGTCTTGT ACCGACTGAA ACATATTCCA 750

GTTGCTGACT TCCCCACAGC ATTTCTGATA CTACCTTGAT AAACGGTTTG 800

GGCCGCAATA CCAATGGTCC GGCTGATGCT GCTCTTGCTG TGATCAATGT 850

-continued

TGACGCTGGC AAACGGTGTG TCCAGATTAC TATACTCCCC ATGACGTCTC 900

AATGCTGATG TGTACTACTT CCAGGTACCG TTTCCGTCTT GTTTCCATAT 950

CCTGTGACCC CAATTGGGTA TTCTCGATTG ACAACCATGA CTTTACGGTC 1000

ATTGAAGTCG ATGGTGTTAA CAGTCAACCT CTCAACGTCG ATTCTGTTCA 1050

GATCTTCGCC GGACAACGTT ACTCGTTCGT 1080

encoding the enzyme laccase C, having the partial translated protein sequence (SEQ ID No. 12)

AIGPVADLHI TDDTIAPDGF SRPAVLAGGG FPGPLITGNK GDAFKLNVID 50

ELTDASMLKX TSIHWHGFFQ KGTNWADGPA FVNQCPITTG NSFLYDFQVP 100

DQAGTYWYHS HLSTQYCDGL RGAFVVYDPS DPHKDLYDVD DESIVITLAD 150

WYHTLARQIV GVAISDTTLI NGLGRNTNGP ADAALAVINV DAGKRYRFRL 200

VSISCDPNWV FSIDNHDFTV IEVDGVNSQP LNVDSVQIFA GQRYSF 246

G. *Cerrena* laccase D1 gene from CBS154.29 strain (SEQ ID No. 13)

GATTCTAATA GACCAGGCAT ACCAAGAGAT CTACAGGTTG ACAGACCATT 50

CTTCTAGGCG GCATTTATGC TGTAGCGTCA GAAATTATCT CTCCATTTGT 100

ATCCCACAGG TCCTGTAATA ACACGGAGAC AGTCCAAACT GGGATGCCTT 150

TTTTCTCAAC TATGGGCGCA CATAGTCTGG ACGATGGTAT ATAAGACGAT 200

GGTATGAGAC CCATGAAGTC AGAACACTTT TGCTCTCTGA CATTTCATGG 250

TTCACACTCT CGAGATGGGA TTGAACTCGG CTATTACATC GCTTGCTATC 300

TTAGCTCTGT CAGTCGGAAG CTATGCTGCA ATTGGGCCCG TGGCCGACAT 350

ACACATTGTC AACAAAGACC TTGCTCCAGA TGGCGTACAA CGTCCAACCG 400

TGCTTGCCGG AGGCACTTTT CCTGGGACGT TGATCACCGG TCAGAAAGTA 450

AGGGATATTA GTTTGCGTCA AAGAGCCAAC CAAAACTAAC CGTCCCGTAC 500

TATAGGGTGA CAACTTCCAG CTCAATGTCA TCGATGATCT TACCGACGAT 550

CGGATGTTGA CGCCAACTTC CATTGTGAGC CTATTATTGT ATGATTTATC 600

CGAATAGTTT CGCAGTCTGA TCATTGGATC TCTATCGCTA GCATTGGCAC 650

GGTTTCTTCC AGAAGGGAAC CGCTTGGGCC GACGGTCCCG CCTTCGTAAC 700

TCAGTGCCCT ATAATAGCAG ATAACTCTTT TCTGTATGAC TTCGACGTCC 750

CAGACCAAGC TGGTACTTTC TGGTATCATA GTCATCTATC CACTCAGTAC 800

TGTGACGGTT TACGTGGTGC CTTCGTTGTG TACGATCCTA ACGATCCTCA 850

CAAAGACCTA TACGATGTTG ATGACGGTGG GTTCCAAATA TTTGTTCTGC 900

AGACATTGTA TTGACGGTGT TCATTATAAT TTCAGAGAGC ACCGTGATTA 950

CCCTTGCGGA TTGGTACCAT GTTCTCGCCC AGACCGTTGT CGGCGCTGCG 1000

TGAGTAACAC ATACACGCGC TCCGGCACAC TGATACTAAT TTTTTTTTAT 1050

TGTAGCACTC CTGATTCTAC CTTGATCAAC GGGTTAGGCC GTTCACAGAC 1100

CGGACCCGCT GATGCTGAGC TGGCTGTTAT CAGCGTTGAA CATAACAAAC 1150

GGTATGTCAT CTCTACCCAG TATCTTCTCT CCTGCTCTAA TTCGCTGTTT 1200

CACCATAGAT ACCGTTTCCG TTTGGTTTCG ATTTCGTGCG ACCCCAACTT 1250

TACCTTCTCC GTTGATGGTC ATAATATGAC TGTCATCGAA GTCGATGGTG 1300

TCAACACACG ACCCCTGACC GTTGACTCTA TTCAAATCTT CGCCGGACAG 1350

AGGTATTCCT TTGTCGTAAG TTAATCGATA TATTCTCCTT ATTACCCCTG 1400

-continued

TGTAATTGAT GTCAATAGCT CAATGCTAAC CAACCCGAAG ACAATTACTG 1450

GATCCGTGCT ATGCCAAACA TCGGTAGAAA TACAACAACA CTGGACGGAA 1500

AGAATGCCGC TATCCTTCGA TACAAGAATG CTTCTGTAGA AGAGCCCAAG 1550

ACCGTTGGGG GCCCCGCTCA ATCCCCGTTG AATGAAGCGG ACCTGCGTCC 1600

ACTCGTACCT GCTCCTGTGG TATGTCTTGT CGCGCTGTTC CATCGCTATT 1650

TCATATTAAC GTTTTGTTTT TGTCAAGCCT GGAAACGCTG TTCCAGGTGG 1700

CGCAGACATC AATCACAGGC TTAACTTAAC TTTCGTACGT ACACCTGGTT 1750

GAAACATTAT ATTTCCAGTC TAACCTCTCT TGTAGAGTAA CGGCCTCTTC 1800

AGCATCAACA ACGCCTCCTT CACTaATCCT TCGGTCCCCG CCTTATTACA 1850

AATTCTGAGC GGTGCTCAGA ACGCTCAAGA TTTACTTCCA ACGGGTAGTT 1900

ACATTGGCCT TGAACTAGGC AAGGTTGTGG AGCTCGTTAT ACCTCCTCTG 1950

GCAGTTGGAG GACCGCACCC TTTCCATCTT CATGGCGTAA GCATACCACA 2000

CTCCCGCAGC CAGAATGACG CAAACTAATC ATGATATGCA GCACAATTTC 2050

TGGGTCGTCC GTAGTGCAGG TAGCGATGAG TATAACTTTG ACGATGCTAT 2100

CCTCAGGGAC GTCGTRAGCA TTGGAGCGGG GACTGATGAA GTCACAATCC 2150

GTTTCGTGGT ATGTCTCACC CCTCGCATTT TGAGACGCAA GAGCTGATAT 2200

ATTTTAACAT AGACCGACAA TCCGGGCCCG TGGTTCCTCC ATTGCCATAT 2250

TGATTGGCAT TTGGAGGCAG GCCTTGCCAT CGTCTTCGCT GAGGGCATCA 2300

ATCAGACCGC TGCAGCCAAC CCAACACCCC GTACGTGACA CTGAGGGTTT 2350

CTTTATAGTG CTGGATTACT GAATCGAGAT TTCTCCACAG AAGCATGGGA 2400

TGAGCTTTGC CCCAAATATA ACGGGTTGAG TGCGAGCCAG AAGGTCAAGC 2450

CTAAGAAAGG AACTGCTATT TAAACGTGGT CCTAGACTAC GGGCATATAA 2500

GTATTCGGGT AGCGCGTGTG AGCAATGTTC CGATACACGT AGATTCATCA 2550

CCGGACACGC TGGGACAATT TGTGTATAAT GGCTAGTAAC GTATCTGAGT 2600

TCTGGTGTGT AGTTCAAAGA GACAGCCCTT CCTGAGACAG CCCTTCCTGA 2650

GACAGCCCTT CCTGAGACGT GACCTCCGTA GTCTGCACAC GATACTYCTA 2700

AATACGTATG GCAAGATGAC AAAGAGGAGG ATGTGAGTTA CTACGAACAG 2750

AAATAGTGCC CGGCCTCGGA GAGATGTTCT TGAATATGGG ACTGGGACCA 2800

ACATCCGGA 2809

encoding the enzyme laccase D1, having the translated protein sequence (SEQ ID No. 14)

MGLNSAITSL AILALSVGSY AAIGPVADIH IVNKDLAPDG VQRPTVLAGG 50

TFPGTLITGQ KGDNFQLNVI DDLTDDRMLT PTSIHWHGFF QKGTAWADGP 100

AFVTQCPIIA DNSFLYDFDV PDQAGTFWYH SHLSTQYCDG LRGAFVVYDP 150

NDPHKDLYDV DDGGTVITLA DWYHVLAQTV VGAATPDSTL INGLGRSQTG 200

PADAELAVIS VEHNKRYRFR LVSISCDPNF TFSVDGHNMT VIEVDGVNTR 250

PLTVDSIQIF AGQRYSFVLN ANQPEDNYWI RAMPNIGRNT TTLDGKNAAI 300

LRYKNASVEE PKTVGGPAQS PLNEADLRPL VPAPVPGNAV PGGADINHRL 350

NLTFSNGLFS INNASFTNPS VPALLQILSG AQNAQDLLPT GSYIGLELGK 400

VVELVIPPLA VGGPHPFHLH GHNFWVVRSA GSDEYNFDDA ILRDVVSIGA 450

GTDEVTIRFV TDNPGPWFLH CHIDWHLEAG LAIVFAEGIN QTAAANPTPQ 500

AWDELCPKYN GLSASQKVKP KKGTAI 526

-continued

H. *Cerrena* laccase D2 gene from CBS115.075 strain
(SEQ ID No. 15)

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GATCTGGACG ATGGTATATA AGACGATGGT ATGAGACCCA TGAAGTCTGA    50

ACACTTTTGC TCTCTGACAT TTCATGGTTC ATACTCTCGA GATGGGATTG   100

AACTCGGCTA TTACATCGCT TGCTATCTTA GCTCTGTCAG TCGGAAGCTA   150

TGCTGCAATT GGGCCCGTGG CCGACATACA CATTGTCAAC AAAGACCTTG   200

CTCCAGATGG TGTACAACGT CCAACCGTGC TCGCCGGAGG CACTTTTCCT   250

GGGACGTTGA TCACCGGTCA GAAAGTAAGG AATATTAGTT TGCGTCAAAG   300

AGCCAACCAA AATTAACCGT CCCGTCCCAT AGGGTGACAA CTTCCAGCTC   350

AATGTCATTG ATGATCTTAC CGACGATCGG ATGTTGACAC CAACTTCCAT   400

TGTGAGCCTA TTATTGTATG ATTTATCCGT ATAGTTTCTC AGTCTGATCA   450

TTGGCTCTCT ATCGCTAGCA TTGGCACGGT TTCTTCCAGA AGGGAACCGC   500

TTGGGCCGAC GGTCCCGCCT TCGTAACTCA GTGCCCTATA ATAGCAGATA   550

ACTCTTTTCT GTATGACTTC GACGTCCCCG ACCAAGCTGG TACTTTCTGG   600

TATCATAGTC ATCTATCCAC TCAGTACTGT GACGGTTTAC GTGGTGCCTT   650

CGTTGTGTAC GATCCTAACG ATCCTCACAA AGACCTATAC GATGTTGATG   700

ACGGTGGGTT CCAAATACTT GACCAAGAAA CATTATATTG ATAGTATCCA   750

CTCTGATTTT CAGAGAGCAC CGTGATTACC CTTGCGGATT GGTACCATGT   800

TCTCGCCCAG ACCGTTGTCG GCGCTGCGTG AGTAACACAT ACACGCGCTC   850

CGGCACACTG ATACTAATTT TTTATTGTAG CACTCCTGAT TCTACCTTGA   900

TCAACGGGTT AGGCCGTTCA CAGACCGGAC CCGCTGATGC TGAGCTGGCT   950

GTTATCAGCG TTGAACATAA CAAACGGTAT GTCATCTCTA CCCATTATCT  1000

TCTCTCCTGC TTTAATTCGC TGTTTCACCA TAGATACCGA TTCCGTTTGG  1050

TTTCGATTTC GTGCGACCCC AACTTTACCT TCTCCGTTGA TGGTCATAAT  1100

ATGACTGTCA TCGAAGTCGA CGGTGTCAAC ACACGACCCC TGACCGTTGA  1150

CTCTATTCAA ATCTTCGCCG GACAGAGGTA TTCCTTTGTC GTAAGTTAAT  1200

CGATATATTC TCCCTATTAC CCCTGTGTAA TTGATGTCAA CAGCTCAATG  1250

CTAACCAACC CGACGACAAT TACTGGATCC GTGCTATGCC AAACATCGGT  1300

AGAAATACAA CAACACTGGA CGGAAAGAAT GCCGCTATCC TTCGATACAA  1350

GAATGCTTCT GTAGAAGAGC CCAAGACCGT TGGGGGCCCC GCTCAATCCC  1400

CGTTGAATGA AGCGGACCTG CGTCCACTCG TACCTGCTCC TGTGGTATGT  1450

CTTGTCGTGC TGTTCCATCG CTATTTCATA TTAACGTTTT GTTTTTGTCA  1500

AGCCTGGAAA CGCTGTTCCA GGTGGCGCAG ACATCAATCA CAGGCTTAAC  1550

TTAACTTTCG TACGTACACC TGGTTGAAAC ATTATATTTC CAGTCTAACC  1600

TCTTGTAGAG TAACGGCCTT TTCAGCATCA ACAACGCCTC CTTCACTAAT  1650

CCTTCGGTCC CCGCCTTATT ACAAATTCTG AGCGGTGCTC AGAACGCTCA  1700

AGATTTACTT CCAACGGGTA GTTACATTGG CCTTGAACTA GGCAAGGTTG  1750

TGGAGCTCGT TATACCTCCT CTGGCAGTTG GAGGACCGCA CCCTTTCCAT  1800

CTTCATGGCG TAAGCATACC ACACTCCCGC AGCCAGAATG ACGCAAACTA  1850

ATCATGATAT GCAGCACAAT TTCTGGGTCG TCCGTAGTGC AGGTAGCGAT  1900

GAGTATAACT TTGACGATGC TATCCTCAGG GACGTCGTGA GCATTGGAGC  1950
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-continued

GGGGACTGAT GAAGTCACAA TCCGTTTCGT GGTATGTCTC ACCCCTCGCA 2000

TTTTGAGACG CAAGAGCTGA TATATTTTAA CATAGACCGA CAATCCGGGC 2050

CCGTGGTTCC TCCATTGCCA TATTGATTGG CATTTGGAGG CAGGCCTTGC 2100

CATCGTCTTC GCTGAGGGCA TCAATCAGAC CGCTGCAGCC AACCCAACAC 2150

CCCGTACGTG ACACTGAGGG TTTCTTTATA GTGCTGGATT ACTGAATCGA 2200

GATTTCTCCA CAGAAGCATG GGATGAGCTT TGCCCCAAAT ATAACGGGTT 2250

GAGTGCGAGC CAGAAGGTCA AGCCTAAGAA AGGAACTGCT ATTTAAACG 2299

encoding the enzyme laccase D2, having the translated protein sequence (SEQ ID No. 16)

MGLNSAITSL AILALSVGSY AAIGPVADIH IVNKDLAPDG VQRPTVLAGG 50

TFPGTLITGQ KGDNFQLNVI DDLTDDRMLT PTSIHWHGFF QKGTAWADGP 100

AFVTQCPIIA DNSFLYDFDV PDQAGTFWYH SHLSTQYCDG LRGAFVVYDP 150

NDPHKDLYDV DDGGTVITLA DWYHVLAQTV VGAATPDSTL INGLGRSQTG 200

PADAELAVIS VEHNKRYRFR LVSISCDPNF TFSVDGHNMT VIEVDGVNTR 250

PLTVDSIQIF AGQRYSFVLN ANQPDDNYWI RAMPNIGRNT TTLDGKNAAI 300

LRYKNASVEE PKTVGGPAQS PLNEADLRPL VPAPVPGNAV PGGADINHRL 350

NLTFSNGLFS INNASFTNPS VPALLQILSG AQNAQDLLPT GSYIGLELGK 400

VVELVIPPLA VGGPHPFHLH GHNFWVVRSA GSDEYNFDDA ILRDVVSIGA 450

GTDEVTIRFV TDNPGPWFLH CHIDWHLEAG LAIVFAEGIN QTAAANPTPQ 500

AWDELCPKYN GLSASQKVKP KKGTAI 526

I. *Cerrena* laccase E gene (partial) from CBS154.29 strain (SEQ ID No. 17)

TGCAATCGGA CCGGTGGCCG ACCTCAAGAT CGTAAACCGA GACATTGCAC 50

CTGACGGTTT TATTCGTCCC GCCGTTCTCG CTGGAGGGTC GTTCCCTGGT 100

CCTCTCATTA CAGGGCAGAA AGTACGTTAC GCTATCTCGG TGCTTTGGCT 150

TAATTAAACT ATTTGACTTT GTGTTCTCTT AGGGGAACGA GTTCAAAATC 200

AATGTAGTCA ATCAACTGAC CGATGGTTCT ATGTTAAAAT CCACCTCAAT 250

CGTAAGCAGA ATGAGCCCTT TGCATCTCGT TTTATTGTTA ATGCGCCCAC 300

TATAGCATTG GCATGGATTC TTCCAGAAGG GAACAAACTG GGCAGACGGT 350

CCTGCGTTCG TGAACCAATG TCCAATCGCC ACGAACAATT CGTTCTTGTA 400

TCAGTTTACC TCACAGGAAC AGCCAGGTGA GTATGAGATG GAGTTCATCC 450

GAGCATGAAC TGATTTATTT GGAACCTAGG CACATTTTGG TACCATAGTC 500

ATCTTTCCAC ACAATACTGC GATGGTTTGC GAGGGCCACT CGTGGTGTAT 550

GACCCACAAG ACCCGCATGC TGTTCTCTAC GACGTCGACG ATGGTTCGTA 600

CTTCGCATAT CCACGCTCGC TTTCATACAA TGTAAACTTT GTTCCTCCAG 650

AAAGTACAAT CATCACGCTC GCGGATTGGT ATCATACCTT GGCTCGGCAA 700

GTGAAAGGCC CAGCGTAAGG CACTTTAGTG TTTCCTCATA GTCCAAGAAA 750

TTCTAACACG CCTTCTTCAT CAGGGTTCCT GGTACGACCT TGATCAACGG 800

GTTGGGGCGT CACAACAATG GTCCTCTAGA TGCTGAACTA GCGGTGATCA 850

GTGTTCAAGC CGGCAAACGG CAAGTTCAAT TCACACTTTT CACTCTGTAC 900

CTTCTTCCTG ACATTCTTTT CTTGTAGTTA CCGCTTCCGC CTGATTTCAA 950

TTTCATGCGA TCCCAACTAC GTATTCTCCA TTGATGGCCA TGATATGACT 1000

GTCATCGAAG TGGATAGTGT TAACAGTCAA CCTCTCAAGG TAGATTCTAT 1050

-continued

CCAAATATTT GCAGGTCAGA GATATTCGTT CGTGGTGAGT CAGATCAGGG 1100

CATATCCTTT TGTCGATACG TCATTGACCA TATAATGCTA CAAGCTGAAT 1150

GCCAACCAAC CAG 1163

encoding the enzyme laccase E, having the partial translated protein sequence (SEQ ID No. 18)

AIGPVADLKI VNRDIAPDGF IRPAVLAGGS FPGPLITGQK GNEFKINVVN 50

QLTDGSMLKS TSIHWHGFFQ KGTNWADGPA FVNQCPIATN NSFLYQFTSQ 100

EQPGTFWYHS HLSTQYCDGL RGPLVVYDPQ DPHAVLYDVD DESTIITLAD 150

WYHTLARQVK GPAVPGTTLI NGLGRHNNGP LDAELAVISV QAGKRQVQFT 200

LFTLYRFRLI SISCDPNYVF SIDGHDMTVI EVDSVNSQPL KVDSIQIFAG 250

QRYSFVLNAN QP 262

The term "% identity" herein and refers to the level of nucleic acid or amino acid sequence identity between the nucleic acid sequence that encodes a laccase described herein or the laccase amino acid sequence, when aligned using a sequence alignment program.

For example, as used herein, 80% sequence identity is determined by an algorithm, and accordingly a homologue of a given sequence has greater than 80% sequence identity over a length of the given sequence. Exemplary levels of sequence identity include, but are not limited to, 80, 85, 90, 95, 98% or more sequence identity to a given sequence, e.g., the coding sequence for a laccase, as described herein.

Exemplary computer programs which can be used to determine identity between two sequences include, but are not limited to, the suite of BLAST programs, e.g., BLASTN, BLASTX, and TBLASTX, BLASTP and TBLASTN, publicly available on the Internet at www.ncbi.nlm.nih.gov/BLAST. See also, Altschul, et al., 1990 and Altschul, et al., 1997.

Sequence searches are typically carried out using the BLASTN program when evaluating a given nucleic acid sequence relative to nucleic acid sequences in the GenBank DNA Sequences and other public databases. The BLASTX program is preferred for searching nucleic acid sequences that have been translated in all reading frames against amino acid sequences in the GenBank Protein Sequences and other public databases. Both BLASTN and BLASTX are run using default parameters of an open gap penalty of 11.0, and an extended gap penalty of 1.0, and utilize the BLOSUM-62 matrix. (See, e.g., Altschul, et al., 1997.)

An alignment of selected sequences in order to determine "% identity" between two or more sequences, may be performed using, for example, the CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix.

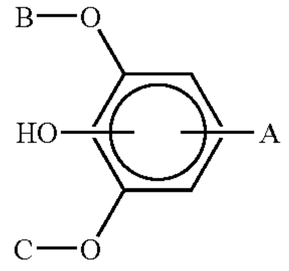
II. Mediators

In an embodiment, the enzymatic oxidation system further comprises one or more chemical mediator agents which enhance the activity of the laccase enzyme. The term "chemical mediator" (or "mediator" may be used interchangeably herein) is defined herein as a chemical compound which acts as a redox mediator to effectively shuttle electrons between the enzyme exhibiting oxidase activity and the dye. Chemical mediators are also known as enhancers and accelerators in the art.

The chemical mediator may be a phenolic compound, for example, methyl syringate, and related compounds, as described in WO 95/01426 and 96/12845. The chemical mediator may also be an N-hydroxy compound, an N-oxime compound, or an N-oxide compound, for example, N-hydroxybenzotriazole, violuric acid, or N-hydroxyacetanilide. The chemical mediator may also be a phenoxazine/phenothiazine compound, for example, phenothiazine-10-propionate. The chemical mediator may further be 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). Other chemical mediators are well known in the art. For example, the compounds disclosed in WO 95/01426 are known to enhance the activity of a laccase. In particular embodiments, the mediator may be acetosyringone, methyl syringate, ethyl syringate, propyl syringate, butyl syringate, hexyl syringate, or octyl syringate.

Preferably, the mediator is 4-cyano-2,6-dimethoxyphenol, 4-carboxamido-2,6-dimethoxyphenol or an N-substituted derivative thereof such as, for example, 4-(N-methyl carboxamido)-2,6-dimethoxyphenol, 4-[N-(2-hydroxyethyl) carboxamido]-2,6-dimethoxyphenol, or 4-(N,N-dimethyl carboxamido)-2,6-dimethoxyphenol.

The mediator used in the present invention may be described by the following formula:



in which formula A is a group such as —R, -D, —CH═CH-D, —CH═CH—CH═CH-D, —CH═N-D, —N═N-D, or —N═CH-D, in which D is selected from the group consisting of —CO-E, —SO_2-E, —CN, —NXY, and —N^+XYZ, in which E may be —H, —OH, —R, —OR, or —NXY, and X and Y and Z may be identical or different and selected from —H, —OH, —OR and —R; R being a C_1-C_{16} alkyl, preferably a C_1-C_8 alkyl, which alkyl may be saturated or unsaturated, branched or unbranched and optionally substituted with a carboxy, sulfo or amino group; and B and C may be the same or different and selected from $C_m H_{2m+1}$; $1 \leqq m \leqq 5$.

In an embodiment A in the above mentioned formula is —CN or —CO-E, in which E may be —H, —OH, —R, —OR, or —NXY, where X and Y may be identical or different and selected from —H, —OH, —OR and —R, R being a C_1-C_{16} alkyl, preferably a C_1-C_8 alkyl, which alkyl may be

saturated or unsaturated, branched or unbranched and optionally substituted with a carboxy, sulfo or amino group; and B and C may be the same or different and selected from $C_m H_{2m+1}$; $1 \leqq m \leqq 5$.

In the above mentioned formula A may be placed meta to the hydroxy group instead of being placed in the para-position as shown.

In particular embodiments, the mediator may be acetosyringone, methylsyringate, ethylsyringate, propylsyringate, butylsyringate, hexylsyringate, or octylsyringate. Preferably, the mediator is 4-cyano-2,6-dimethoxyphenol, 4-carboxamido-2,6-dimethoxyphenol or a N-substituted derivative thereof such as 4-(N-methyl carboxamido)-2,6-dimethoxyphenol, 4-[N-(2-hydroxyethyl)carboxamido]-2,6-dimethoxyphenol, or 4-(N,N-dimethyl carboxamido)-2,6-dimethoxyphenol.

The mediator of the invention may be present in concentrations of from 0.005-1000 μmole per g denim, preferably 0.05-500 μmole per g denim, more preferably 0.5-100 μmole per g denim.

The mediators may be prepared by methods known to the skilled artisan, such as those disclosed in WO 97/11217, WO 96/12845 and U.S. Pat. No. 5,752,980.

III. Utility

Industrial applications of laccases include bleaching of pulp and paper and textile bleaching, for example, of indigo-dyed denim fabrics. Laccases have also been found to be useful for hair dyeing (see, e.g., WO 95/33836 and WO 95/33837). European Patent No. 0504005 discloses that laccases can be used for dyeing wool.

The laccases described herein find use in the dyeing and bleaching of textiles, fibers, yams and the like. The laccases also find use in the treatment of waste water, the delignification of pulp, the depolymerization of high molecular weight aggregates, deinking waste paper, the polymerization of aromatic compounds, radical mediated polymerization and cross-linking reactions (e.g., paints, coatings, biomaterials), and the activation of dyes and to couple organic compounds. The laccases may be used in a cleaning composition or component thereof, or in a detergent.

As described herein, the laccases are capable of oxidizing a wide variety of colored compounds having different chemical structures, using oxygen as the electron acceptor. Accordingly, the laccases presented herein can be used in applications where it is desirable to modify the color associated with colored compounds, such as in cleaning, e.g., for removing the food stains on fabric. In certain situations, a mediator or enhancer can be used to obtain desirable effects.

The laccases presented herein can be used in the field of textiles. For example, the laccases described herein can be used in the treatment, processing, finishing, polishing, or production of fibers, or other fabrics or articles of manufacture. The enzymes herein can be useful, for example, in denim treatment (bleaching work-up processes); in de-coloring indigo waste; in fabric dyeing; in textile bleaching processes; in fiber modification; in achieving enhanced fiber or fabric properties; etc.

The laccases described herein can be used in the leather industry. For example, the laccases can be used in the processing of animal hides including but not limited to de-hairing, liming, bating and/or tanning of hides.

Also disclosed herein is a process for the removal of lignin from lignocellulose-containing material, the bleaching of lignocellulose-containing material (i.e. the enzymatic de-inking of recycled paper) and/or the treatment of waste water arising from the manufacture of paper or cellulose. The process uses laccase enzymes obtained from *Cerrena* sp., at the same time adding or metering in non-aromatic redox agents plus phenolic and/or non-phenolic aromatic redox compounds, the phenolic and non-phenolic units of the lignin either being oxidized directly by the action of these phenolic and/or non-phenolic aromatic compounds, or the lignin being oxidized by other phenolic and/or non-phenolic compounds produced by the oxidizing action of these compounds.

The laccases described herein can be used in the field of pulp and paper. For example, the laccases can be used in the manufacture of paper pulps and fluff pulps from raw materials such as wood, bamboo, and cereal rice straw; the manufacture of paper and boards for printing and writing, packaging, sanitary and other technical uses; recycling of cellulose fiber for the purpose of making paper and boards; and the treatment of waste products generated by and treated at pulp or paper mills and other facilities specifically dedicated to the manufacture of paper, pulp, or fluff. The enzymes presented herein can be useful, for example, in wood processing; in pulp bleaching; in wood fiber modification; in bio-glue (lignin activation) for MDF manufacturing; for enhanced paper properties; in ink removal; in paper dyeing; in adhesives (e.g. lignin based glue for particle- or fiber boards); etc.

The laccases described herein can be used in the field of feed. For example, the laccases presented herein can be used as a feed additive alone or as part of a feed additive with the aim to increase the nutritional value of feed for any kind of animals such as chicken, cows, pigs, fish and pets; and/or as a processing aid to process plant materials and food industry by products with the aim to produce materials/products suitable as feed raw materials.

The laccases described herein can be used in the field of contact lens cleaning. For example, the laccases can be used in the cleaning, storage, disinfecting, and/or preservation of contact lens.

The laccases described herein can be used in the field of starch. For example, the laccases can be used in the processing of a substrate including starch and/or grain to glucose (dextrose) syrup, fructose syrup or any other syrup, alcohol (potable or fuel) or sugar. Such starch processing may include processing steps such as liquefaction, saccharification, isomerization, and de-branching of a substrate.

The laccases described herein can be used in the field of food. For example, the laccases can be used in the preparation, processing, or as an active ingredient in foods such as yellow fat, tea based beverages, culinary products, bakery, and frozen foods for human consumption. The laccases can be used, for example, as a bread improver, in food preservation, as an oxygen scavenger, etc.

The laccases described herein can be used in the field of personal care. For example, the laccases can be used in the preparation of personal products for humans such as fragrances, and products for skin care, hair care, oral hygiene, personal washing and deodorant and/or antiperspirants, for humans. The enzymes presented herein can be useful, for example, in hair dyeing and/or bleaching, nails dyeing and/or bleaching; skin dyeing and/or bleaching; surface modification (e.g., as coupling reagent); as an anti-microbial agent; in odor removal; teeth whitening; etc.

The laccases described herein can be used in the field of cleaning. For example, the laccases can be used in the cleaning, treatment or care of laundry items such as clothing or fabric; in the cleaning of household hard surfaces; in dishcare, including machine dishwashing applications; and in soap bars and liquids and/or synthetic surfactant bars and liquids. The enzymes presented herein can be useful, for example, in stain removal/de-colorization, and/or in the removal of odors, and/or in sanitization, etc.

The laccases described herein can be used in the field of waste-water treatment. For example, the laccases can be used in decolorization of colored compounds; in detoxification of phenolic components; for anti-microbial activity (e.g., in water recycling); in bio-remediation; etc.

The laccases described herein can be used in the field of bio-materials. For example, the laccases can be used as bio-catalysts for various organic reactions; and/or in connection with biopolymers; in connection with packaging; in connection with adhesives; in surface modification (activation and coupling agent); in production of primary alcohols; in connection with biosensors and/or organic syntheses; etc.

The laccases described herein can be used in the field of anti-microbials. For example, the laccases can be used as an anti-microbial agent in cleaning compositions, or for reducing or eliminating the microbial load of various foods (e.g., meats) or feed.

The laccase mediators can be used as sanitization and antimicrobial agents (e.g., wood protection, detergents). The mediators may be used independently of the enzymes or in conjunction with the enzymes.

As used herein, "cleaning compositions" and "cleaning formulations" refer to compositions that find use in the removal of undesired compounds from items to be cleaned, such as fabric, etc. The term encompasses any materials/compounds selected for the particular type of cleaning composition desired and the form of the product (e.g., liquid, gel, granule, or spray composition), as long as the composition is compatible with the laccase and other enzyme(s) used in the composition. The specific selection of cleaning composition materials are readily made by considering the surface, item or fabric to be cleaned, and the desired form of the composition for the cleaning conditions during use.

The terms further refer to any composition that is suited for cleaning and/or bleaching any object and/or surface. It is intended that the terms include, but are not limited to detergent compositions (e.g., liquid and/or solid laundry detergents and fine fabric detergents; hard surface cleaning formulations, such as for glass, wood, ceramic and metal counter tops and windows; carpet cleaners; oven cleaners; and textile and laundry pre-spotters, as well as dish detergents).

Indeed, the term "cleaning composition" as used herein, includes unless otherwise indicated, granular or powder-form all-purpose or heavy-duty washing agents, especially cleaning detergents; liquid, gel or paste-form all-purpose washing agents, especially the so-called heavy-duty liquid (HDL) types; liquid fine-fabric detergents; hand dishwashing agents or light duty dishwashing agents, especially those of the high-foaming type; machine dishwashing agents, including the various tablet, granular, liquid and rinse-aid types for household and institutional use; liquid cleaning and disinfecting agents, car or carpet shampoos, bathroom cleaners; hair shampoos and hair-rinses; shower gels and foam baths and metal cleaners; as well as cleaning auxiliaries such as bleach additives and "stain-stick" or pre-treat types.

As used herein, the terms "detergent composition" and "detergent formulation" are used in reference to mixtures which are intended for use in a wash medium for the cleaning of soiled objects. In some embodiments, the term is used in reference to laundering fabrics and/or garments (e.g., "laundry detergents"). In alternative embodiments, the term refers to other detergents, such as those used to clean dishes, cutlery, etc. (e.g., "dishwashing detergents"). It is not intended that the presently contemplated compositions be limited to any particular detergent formulation or composition. Indeed, it is intended that in addition to laccase, the term encompasses detergents that contain surfactants, transferase(s), hydrolytic enzymes, builders, bleaching agents, bleach activators, bluing agents and fluorescent dyes, caking inhibitors, masking agents, enzyme activators, antioxidants, and solubilizers.

As used herein the term "hard surface cleaning composition," refers to detergent compositions for cleaning hard surfaces such as floors, walls, tile, stainless steel vessels (e.g., fermentation tanks), bath and kitchen fixtures, and the like. Such compositions are provided in any form, including but not limited to solids, liquids, emulsions, etc.

EXAMPLES

Example 1

Amino Acid Sequence Analysis of *Cerrena unicolor* Laccase

Four Peptide sequences were obtained using a commercially available laccase: AIGPVADLHI (SEQ ID No. 19), MLTPTSI (SEQ ID No. 20), TVGGPA (SEQ ID No. 21) and YSFVLNANQP (SEQ ID No. 22). The commercially available laccase was purified. N-terminal sequencing resulted in SEQ ID No. 19. Proteolytic digestion with trypsin of the purified sample was performed. Fragments were separated by gel electrophoresis with 3 bands selected and collected manually. Peptide sequencing was performed for each band and resulted in SEQ ID Nos. 20, 21 and 22.

Example 2

a. Cloning of *Cerrena unicolor* Laccase A Gene from ATCC20013 Strain

To clone the laccase A gene from ATCC 20013 strain, two primers were designed and obtained from Invitrogen: TTCGCAGGTCAACGATATTC (SEQ ID No. 35) based on DNA sequence of the laccase B gene obtained from ATCC20013 strain (see example 3a) and GTTAGGTGGTTGAAGGATTG (SEQ ID No. 36) based on laccase A gene obtained from CBS115.075 strain (see example 2c). The primers were used in a highT PCR reaction containing genomic DNA obtained from ATCC 20013 strain as template (see example 3). The PCR fragment was purified using a QIAquick spin column from Qiagen and cloned into pTOPO plasmid using TOPO cloning kit (Invitrogen). Twenty-two clones were amplified using Ready-To-Go PCR beads (GE Healthcare) and three PCR fragments (2-1, 2-3 and 2-6) were sequenced. 1316 bps DNA sequence of the laccase A gene from ATCC20013 is listed as SEQ ID No 37.

b. Cloning of *Cerrena unicolor* Laccase A Gene from CBS154.29 Strain

To clone the laccase A gene from CBS154.29 strain, two primer was designed and obtained from Invitrogen: CACCAGCATGAGCTCAAAGCTAC (SEQ ID No. 45) based on laccase A gene obtained from CBS115.075 strain (see example 2c) and primer of the SEQ ID No. 36. The primers were used in a Herculase PCR reaction containing genomic DNA template obtained from CBS154.29 strain, dNTPs, primer and 4% DMSO in 1× buffer. The PCR mixture was heated to 98° C. for 4 minutes to denature the DNA template. Herculase® II enzyme (Stratagene) was added to the tube and PCR reaction was performed in 30 cycles of 98° C. for 30 seconds, 50° C. for 30 seconds and 72° C. for 2 minute. The final extension at 72° C. was done for 5 minutes and the reaction was chilled to 4° C. The PCR fragment was purified using the QIAquick spin column and cloned into pENTR/D-TOPO vector (Invitrogen). Fifteen clones were amplified using Ready-To-Go PCR beads and plasmids were isolated

from two clones (pENTR15-24 and pENTR15-30) and the DNA templates were sequenced. 2374 bps DNA sequence of the laccase A gene from CBS154.29 was obtained. The DNA sequence is listed as SEQ ID No. 3 and the translated protein sequence is listed as SEQ ID No. 4.

c. Cloning of *Cerrena unicolor* Laccase A Gene from CBS115.075 Strain

The primer CAATCTATGACCGTAGATTC (SEQ ID No. 39) based on the laccase B gene from ATCC20013 strain (see example 3a) and primer NNNNNNNNNNCGATCG (SEQ ID No. 38) where N represents a mixture of all four nucleotides (A, T, C and G) were used in lowT PCR reaction (see example 3a). Genomic DNA was extracted from *Cerrena unicolor* strain (CBS115.075) and was used as template in the first round of lowT PCR reaction. The PCR fragments were purified with a QIAquick spin column and used as template in the second round of lowT PCR reaction with primers of SEQ ID No. 35 based on the laccase B gene from ATCC20013 strain (see example 3a) and primer of the SEQ ID No. 38. The PCR fragments were cloned into pTOPO plasmid using TOPO cloning kit. Sixteen clones were amplified using Ready-To-Go PCR beads and three cloned PCR fragments (B2#1, B2#4 and B2#11) were sequenced.

To clone the 3' end of laccase A gene, the primer ACCGTGGTTCCTCCATTGCC (SEQ ID No. 40) and primer of SEQ ID No. 31 were used in the lowT PCR reaction with the genomic DNA extracted from *Cerrena unicolor* strain (CBS115.075) as template in the first round of lowT PCR reaction. The PCR fragments were purified with a QIAquick spin column and used as template in the second round of lowT PCR reaction with primers GACTGGCACTTGGAAGCGGG (SEQ ID No. 41) and primer of SEQ ID No. 31. The PCR fragments were cloned into pTOPO plasmid using TOPO cloning kit. Twenty-two clones were amplified using Ready-To-Go PCR beads and one cloned PCR fragment (D2#2) was sequenced.

To clone the 5' end of the laccase A gene, a primer, GGACCAAGCTGGTACTTTC (SEQ ID No. 42), was designed based on the laccase B gene sequence. It was used to amplify a DNA fragment with primer of SEQ ID No. 36. The genomic DNA extracted from *Cerrena unicolor* strain (CBS115.075) was used as the PCR template. The 1.7 kb PCR fragment was obtained, purified with a QIAquick spin column and cloned into pTOPO plasmid using TOPO cloning kit. Twenty-two clones were analyzed using Ready-To-Go PCR beads. Plasmid DNA from clone (C5#20) was sequenced. To further clone the 5' of laccase A gene, the primer CGTGGTACCAGTCTGCCAGGG (SEQ ID No. 43) and primer of SEQ ID No. 31 were used in the lowT PCR reaction with the genomic DNA extracted from *Cerrena unicolor* CBS115.075 strain as template. From the first round of lowT PCR reaction, the PCR fragment was purified with a QIAquick spin column and used as template in the second round of lowT PCR reaction with primers GGCAGCATCAGTCACGGTCAG (SEQ ID No. 44) and primer of SEQ ID No. 31. The PCR fragment (a3) was amplified again and used as template in a third round of lowT PCR reaction with primers GGCAGCATCAGTCACGGTCAG (SEQ ID No. 44) and primer of SEQ ID No. 31. The PCT fragment (a3-2) was cloned into pTOPO plasmid using TOPO cloning kit. Eleven clones were amplified using Ready-To-Go PCR beads and two cloned PCR fragments (a3-2#10 and a3-2#11) were sequenced. The DNA sequence of the laccase A gene from CBS115.075 strain including the sequence of 5' and 3' of the coding region is listed as SEQ ID No. 1 and the translated protein sequence is listed as SEQ ID No. 2.

Example 3

a. Cloning and Sequencing of the *Cerrena unicolor* Laccase B Gene from ATCC20013 Strain

To clone the DNA fragment encoding the Cerrena laccase gene, four degenerated primers were designed based on the peptide sequence AIGPVADLHI (SEQ ID No. 19) and obtained from Invitrogen. They are named as

```
primerA
GCAATCGGACCNGTNGCAGA;     (SEQ ID No. 23)

primerB
GCAATCGGACCNGTNGCTGA;     (SEQ ID No. 24)

primerC
GCAATCGGACCNGTNGCGGA      (SEQ ID No. 25)
and

primerD
GCAATCGGACCNGTNGCCGA.     (SEQ ID No. 26)
```

Two degenerated primers were designed based on the peptide sequence YSFVLNANQP (SEQ ID No. 22) and obtained from Invitrogen. They are named as

```
primerE
GGTTGATTTGCATTNAGNAC      (SEQ ID No. 27)
and

primerF
GGTTGATTTGCGTTNAGNAC      (SEQ ID No. 28)
```

where N represents a mixture of all four nucleotides (A, T, C and G). The genomic DNA was extracted from ATCC20013 strain and used as template in the lowT PCR reaction contain following combination of primers: PCR reaction 1 contains no DNA and no primer; PCR reaction 2 contains primerA and primerE; PCR reaction 3 contains primerB and primerE; PCR reaction 4 contains primerC and primerE; PCR reaction 5 contains primerD and primerE; PCR reaction 6 contains primerA and primerF; PCR reaction 7 contains primerB and primerF; PCR reaction 8 contains primerC and primerF and PCR reaction 9 contains primerD and primerF. The PCR reaction mixture contained DNA template, primers, 1× buffer, 0.2 mM dNTP and 1 unit of Taq DNA polymerase. The PCR reaction was performed in 30 cycles of 95° C. for 1 minute, 45° C. for 1 minute and 68° C. for 1 minute. The final extension at 72° C. was done for 7 minutes and the reaction was chilled to 4° C. The PCR fragments from reaction 4, 5 and 8 were cut out of a 1.2% agarose gel and pooled. The PCR fragments were extracted from gel with a Qiagen spin column and cloned into pTOPO plasmid using TOPO cloning kit. Thirty-two cloned PCR fragments were selected and sequenced using Ready-To-Go PCR beads and DNA sequence of clone #A30 was identified as laccase B gene.

To clone the 5' end of laccase gene, a primer was designed and obtained from Invitrogen: GGACGTGGCCTTGAGCATAC (SEQ ID No. 29). It was used in first round of lowT PCR reaction with a degenerated oligo NNNNNNNNNNGGATCC (SEQ ID No. 31) where N represents a mixture of all four nucleotides (A, T, C and G). The PCR product was purified using a QIAquick spin column and used as template in a second lowT PCR reaction containing a primer TCTGTCAAGTCGTCAATCAC (SEQ ID No. 30) and primer of SEQ ID No. 31. The PCR fragment was purified using a QIAquick spin column and diluted 1:10 and 1:100 and used as template in the first round of highT PCR reaction performed in 30 cycles of 95° C. for 1 minute, 50° C. for 1 minute and 72°

C. for 1 minute with two primers (SEQ ID No. 30 and SEQ ID No. 31). The final extension at 72° C. was done for 7 minutes and the reaction was chilled to 4° C. The PCR fragment was purified with a QIAquick spin column and used in the second round of highT PCR reaction with primers of TTACCACGAATCAGAGGACC (SEQ ID No. 32) and SEQ ID No. 31. The PCR fragment (D13) was sequenced.

To clone the 3' end of the laccase B gene, a primer was designed and obtained from Invitrogen: CCTCACCTGTATTGGCACAG (SEQ ID No. 33) and used with primer of SEQ ID No. 31 in a first round of lowT PCR reaction. The PCR fragment was purified in a QIAquick spin column and used as template in second round of lowT PCR reaction with primer TTGGTATCATGCCCTTGCTC (SEQ ID No. 34) and primer of SEQ ID No. 31. The PCR fragment was cloned into a pTOPO plasmid using TOPO cloning kit. Sixteen clones were amplified using Ready-To-Go PCR beads and four cloned PCR fragments (C3, C4, C5 and C7) were sequenced.

1337 bps DNA fragment was obtained. The DNA sequence is listed as SEQ ID No. 9 and translated protein sequence is listed as SEQ ID No. 10.

b. Cloning of *Cerrena unicolor* Laccase B Gene from CBS154.29 Strain

Two primers were designed and obtained from Invitrogen:

```
CACCGCGATGTCTCTTCTTCGTAG      (SEQ ID No. 46)
and

TGRAGRTGGAASGGATGWGGTCC       (SEQ ID No. 47)
```

where R represent mixture of nucleotides A and G, S represent mixture of nucleotides C and G, and W represent mixture of nucleotides A and T. The two primers were used in the highT PCR reaction. The PCR fragment (A3) was purified using a QIAquick spin column. The PCR fragment was cloned into pTOPO plasmid using TOPO cloning kit. Sixteen clones were amplified using Ready-To-Go PCR beads and two PCR fragments (A3#1 and A3#5) were sequenced.

To clone the 3' end of the laccase B gene from CBS154.29 strain, a primer was designed and obtained from Invitrogen: GTCCCTGTACTACTCCAGATCC (SEQ ID No. 48) and used with a primer having SEQ ID No. 31 in first round of lowT PCR reaction. The PCR fragment was purified in a QIAquick spin column and used as template in second round of lowT PCR reaction with primer CCAGCAGGAAGCGTGATCGAAC (SEQ ID No. 49) and primer of SEQ ID No. 31. The PCR fragment was cloned into pTOPO plasmid using TOPO cloning kit. Sixteen clones were amplified using Ready-To-Go PCR beads and three PCR fragments (7#6, 7#7 and 7#8) were sequenced. 2663 bps of the laccase B DNA sequence of the CBS154.29 strain is listed as SEQ ID No. 7 and translated protein sequence is listed as SEQ ID No. 8.

c. Cloning of *Cerrena unicolor* Laccase B Gene from CBS115.075 Strain

A primer was designed and obtained from Invitrogen: GTAATCATGTATCACCTGGGCTCAAGG (SEQ ID No. 50). The primer was used in the Herculase PCR reaction (see Example 2b) with primer of SEQ ID No. 46. The PCR fragment was purified using a QIAquick spin column. The PCR fragment was cloned into pTOPO plasmid using TOPO cloning kit. Seventeen clones were analyzed using Ready-To-Go PCR beads and the PCR fragments from four clones (#1, #2, #4 and #5) were sequenced. The plasmid DNA was prepared from two clones (pENTR-laccaseB CBS115075#1 and pENTR-laccaseB CBS115075#3) and both plasmids were sequenced. 2173 bps of the laccase B DNA sequence of the CBS115.075 strain is listed as SEQ ID No. 5 and translated protein sequence is listed as SEQ ID No. 6.

Example 4

Cloning of the *Cerrena unicolor* Laccase C Gene from CBS154.29 Strain

A primer ACGAACGAGTANCGTTGNCC (SEQ ID No. 51), where N represents a mixture of all four nucleotides (i.e., A, T, C and G), was designed based on the translated peptide sequence GQRYSFV (SEQ ID No. 52). This peptide is conserved between the laccase A gene and the laccase B gene (see Examples 2 and 3). The primer was obtained from Invitrogen and was used in the lowT reaction with primer of the SEQ ID No. 24. The PCR fragment was purified using a QIAquick spin column. The PCR fragment was cloned into pTOPO plasmid using TOPO cloning kit. Thirty-three clones were analyzed using Ready-To-Go PCR beads and the PCR fragments from four clones (#12, #5a, #19a and #21a) were sequenced. 1080 bps of the laccase C gene sequence from the CBS154.29 strain is listed as SEQ ID No. 11 and translated protein sequence is listed as SEQ ID No. 12.

Example 5

a. Cloning of *Cerrena unicolor* Laccase D Gene from CBS115.075 Strain

To clone the 5' end of the laccase D gene from CBS115.075 strain, a primer was designed based on laccase D gene from CBS154.29 strain (see Example 5b) (AACACGGAGACAGTCCAAAC, SEQ ID No. 62). It was used in the highT PCR reaction with primer of SEQ ID No. 56. The PCR fragment was purified using a QIAquick spin column and sequenced.

To clone the laccase D gene from CBS115.075 strain, two primers (CACCTCTCGAGATGGGATTGAAC, SEQ ID No. 63 and CGTTTAAATAGCAGTTCCTTTC, SEQ ID No. 64) were designed based on the laccase D gene from CBS154.29 strain (see example 5b). The primers were used in a Herculase PCR reaction (see example 2b) with DNA template of the genomic DNA from CBS115.075 strain. The PCR fragment was purified using the QIAquick spin column and cloned into pENTR/D-TOPO vector. Sixteen clones were amplified using Ready-To-Go PCR beads and the PCR fragments generated from four clones were sequenced. The plasmids were isolated from clone #2 (pENTRE-laccaseD#2) and it was sequenced. 2809 bps DNA sequence of the laccase D gene from CBS115.075 was obtained. The DNA sequence is listed as SEQ ID No. 15 and the translated protein sequence is listed as SEQ ID No. 16.

b. Cloning of *Cerrena unicolor* Laccase D Gene from CBS154.29 Strain

A primer, CTGGTTGGTTNGCATTNAG (SEQ ID No. 53), was designed based on the peptide sequence LNANQP (SEQ ID No. 54). The primer was obtained from Invitrogen and used in the lowT PCR reaction with primer of the SEQ ID No. 26. The PCR fragment was purified using a QIAquick spin column and was cloned into pTOPO plasmid using TOPO cloning kit. Eighteen clones were analyzed using Ready-To-Go PCR beads and PCR fragment from a clone was sequenced.

To clone the 3' end of the laccase D gene, a primer (CACACGACCCCTGACCGTTG, SEQ ID No. 55) was designed. The primer was used in the lowT PCR reaction with primer of the SEQ ID No. 31. The PCR fragment was purified using a QIAquick spin column and was cloned into pTOPO

plasmid using TOPO cloning kit. Twenty-four clones were analyzed using Ready-To-Go PCR beads and PCR fragment(s) from a clone were sequenced.

To clone more of the 3' and the 5' ends of the laccase D gene, inverse PCR was used. 0.4 ug of the genomic DNA from the Cerrena CBS154.29 strain was digested with EcoRV restriction enzyme at 37° C. for 1.5 hours. Digested genomic DNA fragments were precipitated with ethanol. The linear DNA fragments were ligated with T4 DNA ligase in 100 ul volume for more than 5 hours. The ligated DNA fragments were heated to 100° C. for 3 minutes and were used as the DNA template in a first round of the highT PCR reaction using two primers (TGACCGGTGATCAACGTCCC, SEQ ID No. 56, and GGCGCAGACATCAATCACAG, SEQ ID No. 57). The PCR fragments were purified using a QIAquick spin column and were used as a DNA template in the second round of the highT PCR reaction using two primers (TCTTCAGCATCAACAACGCC, SEQ ID No. 58 and TCCGGCAAGCACGGTTGG, SEQ ID No. 59). The PCR fragments from second round of PCR reaction were purified using a QIAquick spin column and were sequenced.

To clone more of the 3' end of laccase D gene from CBS154.29 strain, inverse PCR was used. 0.4 ug of the genomic DNA from the Cerrena CBS154.29 strain was digested with SmaI restriction enzyme at 37° C. for 1.5 hours. Digested genomic DNA fragments were precipitated with ethanol. The linear DNA fragments were ligated with T4 DNA ligase in 100 ul volume for more than 5 hours. The ligated DNA fragments were heated to 100° C. for 3 minutes and were used as the DNA template in a first round of highT PCR reaction with primer TCGTCTTCGCTGAGGGCATC, SEQ ID No. 60, and primer of SEQ ID No. 56. The PCR fragments were purified using a QIAquick spin column and were used as DNA template in the second round of the highT PCR reaction using primer (CAGACCGCTGCAGCCAACCC, SEQ ID No. 61) and primer of SEQ ID No. 59. The PCR fragments from the second round of PCR reaction were purified using a QIAquick spin column and cloned into pTOPO plasmid using TOPO cloning kit. Twenty-one clones were analyzed using Ready-To-Go PCR beads and PCR fragment from clones #Ce11 and #Ce14 were sequenced. 2809 bps of the laccase D gene sequence from the CBS154.29.49 strain is listed as SEQ ID No. 13 and the translated protein sequence is listed as SEQ ID No. 14.

Example 6

Cloning of *Cerrena unicolor* Laccase E Gene from CBS154.29 Strain

The primer of SEQ ID No. 53 was used in the lowT PCR reaction with primer of the SEQ ID No. 26 (see Example 5b). The PCR fragment was purified using a QIAquick spin column and was cloned into pTOPO plasmid using TOPO cloning kit. Eighteen clones were analyzed using Ready-To-Go PCR beads and the PCR fragment from clone #Ae17 was sequenced. 1163 bps of the laccase E gene sequence from the CBS154.29.49 strain is listed as SEQ ID No. 17 and the translated protein sequence is listed as SEQ ID No. 18.

Example 7

Expression of Laccase A Gene in *Trichoderma*

To construct the expression plasmid for the laccase A gene of the CBS strain 115.075, two primers (SEQ ID No. 45 and SEQ ID No. 36) were used in the Herculase PCR reaction containing genomic DNA template obtained from 115.075 strain, dNTPs, and 4% DMSO in 1× buffer. The PCR mixture was heated to 98° C. for 4 minutes to denature the DNA template. Herculase® II enzyme (Stratagene) was added to the tube and PCR reaction was performed in 30 cycles of 98° C. for 30 seconds, 50° C. for 30 seconds and 72° C. for 2 minute. The final extension at 72° C. was done for 5 minutes and the reaction was chilled to 4° C. The PCR fragment was purified using the QIAquick spin column and cloned into pENTR/D-TOPO vector. Fifteen clones were amplified using Ready-To-Go PCR beads and plasmid DNA was isolated from pENTR-laccaseA-CBS115.075#11 clone. The laccase A gene portion was sequenced to confirm fidelity of the PCR amplification of the laccase A gene. The plasmid of pENTR-laccaseA-CBS115.075#11 (50 ng) was converted to the expression plasmid pTrex3g-laccaseA (FIG. **1**) in a 10 ul LB clonase II reaction (Invitrogen) containing 6.5 ul of TE, 1 ul of pTrex3g vector (0.1 mg/ml) and 2 ul of ClonaseII. The expression plasmid was confirmed by DNA sequencing and transformed biolistically into a *Trichoderma* strain. Transformation of the *Trichoderma* strain by the biolistic transformation method was accomplished using a Biolistic® PDS-1000/he Particle Delivery System from Bio-Rad (Hercules, Calif.) following the manufacturers instructions (see WO 05/001036 and US 2006/0003408). Sixty-six transformants were selected and were transferred to new plates. A total of 15 stable transformants were grown in 30 ml of the Proflo media for 2 days at 30° C. Five mls of 2 days old culture from Proflo media were transferred to 50 mls of defined media containing 1 mM copper. The cultures were grown for 5 days at 28° C. Culture broths were centrifuged and supernatants were used for ABTS assay.

Example 8

a. Expression of Laccase B Gene in *Aspergillus*

To construct the expression plasmid for the laccase B gene of the CBS strain 115.075, two primers GCAGATCTGCGATGTCTCTTCTTCGTAGCTTGAC (SEQ ID No. 72) and GAGGTCACCTCTAGATCATGTATCACCTGGGCTCAAGGCATC (SEQ ID No. 73) were used in the Herculase PCR reaction containing genomic DNA template obtained from 115.075 strain (see Example 2b). The PCR fragment was purified using the QIAquick spin column and digested with restriction enzyme BglII and XbaI. The DNA fragment was purified again with the QIAquick spin column and was cloned into BglII and XbaI digested pGAPT vector. Fidelity of the plasmid was confirmed by DNA sequencing. The resulting plasmid pKB401 (FIG. **2**) was transformed into *A. niger* 2445 for checking expression of laccase B gene. Thirty-four transformants were selected and were transferred onto MM plates and grew for 4 days at 30° C. A small plug of single colony including spores and mycelium was innoculated on to a CMA plate and grew for 4 days at 30° C. A plug of CMA plate containing confluent spores and mycelium was transferred into to 30 mls of Promosoy special broth (pH6.2) containing 1 mM copper. The cultures were grown for 5 days at 30° C. Culture broths were centrifuged and supernatants were used for ABTS assay.

b. Expression of Laccase B Gene in *Aspergillus* as Fusion to Catalytic Domain of the Glucoamylase

To construct the fusion expression plasmid for the laccase B gene of the CBS strain 115.075, two primers TTGCTAGCAACGTGATCTCCAAGCGTGCAATCGGTCCAGTCACTGACCTAC (51 mer, SEQ ID No. 74) and primer of SEQ ID No. 73 were used in the Herculase PCR reaction containing genomic DNA template obtained from

CBS115.075 strain (see Example 2b). The PCR fragment was purified using the QIAquick spin column and digested with NheI and BstEII and was purified again with the QIAquick spin column. This purified fragment was cloned into NheI and BstEI digested vector pGAMpR2-GV (see US Patent application US20050153399). The resulting plasmid pKB403 (FIG. **3**) was confirmed by sequencing analysis and was transformed into *A. niger* 2445. Twenty-eight transformants were selected and were transferred onto MM plates and grew for 4 days at 30° C. A small plug of single colony including the spores and mycelium were innoculated onto CMA plate and grew for 4 days at 30° C. A plug of CMA plate containing confluent spores and mycelia was transferred into to 30 mls of Promosoy special broth (pH6.2) (see US Patent application US20050153399) containing 1 mM copper. The cultures were grown for 5 days at 30° C. Culture broths were centrifuged and supernatants were used for ABTS assay.

c. Expression of Laccase B Gene in *Trichoderma*

To construct expression plasmid for the laccase B gene of the CBS115.075 strain (see Example 2b). A primer was designed and obtained from Invitrogen: GTAATCATGTATCACCTGGGCTCAAGG (SEQ ID No. 50). The primer was used in the Herculase PCR reaction (see Example 2b) with primer of SEQ ID No. 46. The PCR fragment was purified using the QIAquick spin column and cloned into pENTR/D-TOPO vector (Invitrogen). Seventeen clones were amplified using Ready-To-Go PCR beads and plasmid DNA was isolated from pENTR-CBS115.075#1 clone (see Example 3c). The laccase B gene portion was sequenced to confirm fidelity of the PCR amplification. The plasmid of pENTR-laccaseB-CBS115.075#1 (50 ng) was converted to expression plasmid pTrex3g-laccaseB (see FIG. **1** with the laccase A gene replaced with the laccase B gene) in a 10 ul LB clonase II reaction (Invitrogen) containing 6.5 ul of TE, 1 ul of pTrex3g vector (0.1 mg/ml) and 2 ul of ClonaseII. The expression plasmid was confirmed by DNA sequencing and transformed biolistically into a *Trichoderma* strain. Sixty transformants were selected and were transferred to new plates. A total of 20 stable transformants were grown in 30 ml of the Proflo media for 2 days at 30° C. Three mls of 2 day old culture from Proflo media were transferred to 30 mls of defined media (see US Patent Application 20050153399) containing 1 mM copper. The cultures were grown for 4 days at 28° C. Culture broths were centrifuged and supernatants were used for ABTS assay.

d. Expression of the Laccase B Gene in *Trichoderma* as CBH1 Fusion

To construct the expression plasmid for the laccase B gene of the CBS strain 115.075, a primer was designed and obtained from Invitrogen (GGACTAGTGTCGCCGTTTACAAACGCGCAATCGGTCCAGTCACTGACC, SEQ ID No. 65). The primer was used in combination with the reverse primer (obtained from New England Biolab) in the Herculase PCR reaction containing pENTR-laccaseB CBS115075#1 (see example 3c) as the DNA template. The PCR fragment (SEQ ID No. 66)

```
ACTAGTGTCG CCGTTTACAA ACGCGCAATC GGTCCAGTCA CTGACCTACA     50
TATAGTGAAC CAGAATCTCG ACCCAGATGG TTTCAACCGC CCCACTGTAC    100
TCGCAGGTGG TACTTTCCCC GGTCCTCTGA TTCGTGGTAA CAAGGTACGC    150
TTCATAACCG CCCTCCGTAG ACGTAGGCTT CGGCTGACAT GACCATCATC    200
TGTAGGGAGA TAACTTTAAA ATTAATGTGA TTGACGACTT GACAGAGCAC    250
AGTATGCTCA AGGCTACGTC CATCGTAAGT CCCTGATTAA CGTTTCACCT    300
GGTCATATCG CTCAACGTCT CGAAGCACTG GCATGGGTTC TTCCAGAAGG    350
GAACCAACTG GGCCGATGGC CCCGCCTTTG TCACCCAATG TCCTATCACA    400
TCAGGAAACG CCTTCCTGTA TGATTTCAAC GTTCCGGACC AAGCTGGTAC    450
TTTCTGGTAC CACAGCCATC TCTCTACACA GTATTGTGAC GGTCTTCGTG    500
GTGCCTTTGT CGTCTATGAT CCTAATGATC CCAACAAGCA ACTCTATGAT    550
GTTGATAACG GCAAGTTCCT TGCATATTTC ATTTCTATCA TATCCTCACC    600
TGTATTGGCA CAGAAAGCAC CGTGATTACC TTGGCTGATT GGTATCATGC    650
CCTTGCTCAG ACTGTCACTG GTGTCGCGTG AGTGACAAAT GGCCCTCAAT    700
TGTTCACATA TTTTCCTGAT TATCATATGA TAGAGTATCT GATGCAACGT    750
TGATCAACGG ATTGGGACGT TCGGCCACCG GCCCCGCAAA TGCCCCTCTG    800
GCGGTCATCA GTGTCGAGCG GAATAAGAGG TCAGTTCCAT AATTATGATT    850
ATTTCCCGCG TTACTTCCTA ACAATTATTT TTGTATCCCT CCACAGATAT    900
CGTTTCCGAT TGGTTTCTAT TTCTTGCGAC CCTAACTTTA TTTTCTCAAT    950
TGACCACCAC CCAATGACCG TAATTGAGAT GGACGGTGTT AATACCCAAT   1000
CTATGACCGT AGATTCGATC CAAATATTCG CAGGTCAACG ATATTCATTT   1050
GTCGTAGGTT ATTATAAACT GCCCACCGAT CATCTCTCAC GTAACTGTTA   1100
TAGATGCAAG CCAACCAACC AGTTGGAAAT TATTGGATCC GCGCTAAACC   1150
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-continued

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TAATGTTGGG AACACAACTT TCCTTGGAGG CCTGAACTCC GCTATATTAC  1200

GATATGTGGG AGCCCCTGAC CAAGAACCGA CCACTGACCA AACACCCAAC  1250

TCTACACCGC TCGTTGAGGC GAACCTACGA CCCCTCGTCT ATACTCCTGT  1300

GGTATGTTGT TCTCGTTACA TATACCAAAC CTAATATGAA GACTGAACGG  1350

ATCTACTAGC CGGGACAGCC ATTCCCTGGC GGTGCTGATA TCGTCAAGAA  1400

CTTAGCTTTG GGTTTCGTAC GTGTATTTCA CTTCCCTTTT GGCAGTAACT  1450

GAGGTGGAAT GTATATAGAA TGCCGGGCGT TTCACAATCA ATGGAGCGTC  1500

CCTCACACCT CCTACAGTCC CTGTACTACT CCAGATCCTC AGTGGTACTC  1550

ACAATGCACA GGATCTTCTC CCAGCAGGAA GCGTGATCGA ACTTGAACAG  1600

AATAAAGTTG TCGAAATCGT TTTGCCCGCT GCGGGCGCCG TTGGCGGTCC  1650

TCATCCTTTT CACTTACATG GTGTAAGTAT CAGACGTCCT CATGCCCATA  1700

TTGCTCCGAA CCTTACACAC CTGATTTCAG CACAATTTCT GGGTGGTTCG  1750

TAGCGCCGGT CAAACCACAT ACAATTTCAA TGATGCTCCT ATCCGTGATG  1800

TTGTCAGTAT TGGCGGTGCA AACGATCAAG TCACGATCCG ATTTGTGGTA  1850

TGTATCTCGT GCCTTGCATT CATTCCACGA GTAATGATCC TTACACTTCG  1900

GGTTCTCAGA CCGATAACCC TGGCCCATGG TTCCTTCACT GTCACATTGA  1950

CTGGCATTTG GAGGCTGGGT TCGCTGTAGT CTTTGCGGAG GGAATCAATG  2000

GTACTGCAGC TGCTAATCCA GTCCCAGGTA AGACTCTCGC TGCTTTGCGT  2050

AATATCTATG AATTTAAATC ATATCAATTT GCAGCGGCTT GGAATCAATT  2100

GTGCCCATTG TATGATGCCT TGAGCCCAGG TGATACATGA TTACAAGGGT  2150

GGGCGCGCC                                               2159
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was purified using the QIAquick spin column and digested with restriction enzymes SpeI and AscI. This fragment (SEQ ID No. 66) was then cloned into pTrex4 vector which was also digested with SpeI and AscI to create the expression plasmid (pTrex4-laccaseB, FIG. **4**). The fidelity of the expression plasmid was confirmed by DNA sequencing and transformed biolistically into a *Trichoderma* strain. More than 100 transformants were generated and sixty transformants were transferred to new plates. A total of 20 stable transformants were grown in 30 ml of the Proflo media for 2 days at 30° C. Five mls of 2 days old culture from Proflo media were transferred to 50 mls of defined media containing 1 mM copper. The cultures were grown for 4 days at 28° C. Culture broths were centrifuged and supernatants were used for ABTS assay.

Example 9

a. Expression of Laccase B Gene of the CBS Strain 115.075 in *Streptomyces*

The laccase B protein sequence was used for codon optimization according to *Streptomyces lividans* codon usage. To construct the expression plasmid for the synthesized laccase B gene of the CBS115.075 strain in *Streptomyces*, two primers ACGCAGCCTGAACTAGTTGCGATCCTCTAGAG (SEQ ID No. 75) and CTCTGATCAAGGTCATCAGGTGTCGCCCGGGGACAGG (SEQ ID No. 76) were used in the Herculase PCR reaction containing the optimized DNA template (See Example 2b). The PCR fragment was purified using the QIAquick spin column and was digested with XbaI and BcII. The digested fragment was purified by the QIAquick spin column and was cloned into XbaI and BamHI disgested pKB105 (see US 20060154843). The correctness of the resulting plasmid pKB251 (FIG. **5**) was confirmed by DNA sequencing. The DNA of plasmid pKB251 was transformed into *Streptomyces lividans* g3s3 strain (see US 20060154843). Twelve thiostrepton resistant transformants were picked and transferred into seed shake flask (20 ml of TSG medium containing 50 ug/ml of thiostrepton in DMSO), grown for 2 days at 30° C. Three mls of 2 days old culture from seed shake flask were transferred to 30 mls of *Streptomyces* modified production medium II containing 1 mM copper. The cultures were grown for 4 days at 30° C. Culture broths were centrifuged and supernatants were used for ABTS assay.

Example 10

Expression of the Laccase B Gene in *Trichoderma* as CBH1 Fusion Using codon optimized synthetic gene

The optimized synthtetic laccase B gene (SEQ ID NO:67):

```
ACTAGTGTCG CCGTTTACAA ACGCGCAATC GGTCCCGTCA CTGACCTGCA    50

TATTGTGAAC CAGAATCTCG ACCCCGATGG TTTCAACCGC CCCACTGTCC   100
```

-continued

```
TCGCAGGTGG TACTTTCCCC GGTCCTCTGA TTCGTGGTAA CAAGGGAGAT   150

AACTTTAAAA TTAATGTGAT TGACGACTTG ACAGAGCACA GCATGCTCAA   200

GGCTACGTCC ATCCACTGGC ATGGCTTCTT CCAGAAGGGA ACCAACTGGG   250

CCGATGGCCC CGCCTTTGTC ACCCAATGTC CTATCACATC AGGAAACGCC   300

TTCCTGTACG ATTTCAACGT TCCGGACCAA GCTGGTACTT TCTGGTACCA   350

CAGCCATCTC TCTACACAGT ACTGTGACGG TCTTCGTGGT GCCTTTGTCG   400

TCTACGATCC TAATGATCCC AACAAGCAAC TCTACGATGT TGATAACGGC   450

AACACCGTGA TTACCTTGGC TGATTGGTAC CATGCCCTTG CTCAGACTGT   500

CACTGGTGTC GCAGTCTCTG ATGCAACGTT GATCAACGGA TTGGGACGTT   550

CGGCCACCGG CCCCGCAAAT GCCCCTCTGG CGGTCATCAG CGTCGAGCGC   600

AATAAGCGCT ATCGTTTCCG ATTGGTTTCT ATTTCTTGCG ACCCTAACTT   650

TATTTTCTCA ATTGACCACC ACCCCATGAC CGTCATTGAG ATGGACGGTG   700

TTAATACCCA ATCTATGACC GTAGATTCGA TCCAAATCTT CGCAGGTCAA   750

CGATACTCAT TTGTCATGCA AGCCAACCAA CCAGTTGGAA ATTACTGGAT   800

CCGCGCTAAA CCTAATGTTG GCAACACAAC TTTCCTTGGA GGCCTGAACT   850

CCGCTATCTT GCGATACGTG GGAGCCCCTG ACCAAGAACC GACCACTGAC   900

CAAACACCCA ACTCTACACC GCTCGTTGAG GCGAACCTGC GACCCCTCGT   950

CTACACTCCT GTGCCGGGAC AGCCATTCCC TGGCGGTGCT GATATCGTCA  1000

AGAACTTGGC TTTGGGTTTC AATGCCGGGC GTTTCACAAT CAATGGAGCG  1050

TCCCTCACAC CTCCTACAGT CCCTGTCCTG CTCCAGATCC TCAGCGGTAC  1100

TCACAATGCA CAGGATCTTC TCCCGGCAGG AAGCGTGATC GAACTTGAAC  1150

AGAATAAAGT TGTCGAAATC GTTTTGCCCG CTGCGGGCGC CGTTGGCGGT  1200

CCTCATCCTT TTCACTTGCA TGGTCACAAT TTCTGGGTGG TTCGTAGCGC  1250

CGGTCAAACC ACATACAATT TCAATGATGC TCCTATCCGT GATGTTGTCA  1300

GCATTGGCGG TGCAAACGAT CAAGTCACGA TCCGATTTGT GACCGATAAC  1350

CCTGGCCCAT GGTTCCTTCA CTGTCACATT GACTGGCATT TGGAGGCTGG  1400

ATTCGCTGTC GTCTTTGCGG AGGGAATCAA TGGTACTGCA GCTGCTAATC  1450

CCGTCCCGGC GGCTTGGAAT CAATTGTGCC CGTTGTACGA TGCCTTGAGC  1500

CCGGGTGATA CATGAGGCGC GCC                                1523
```

encoding the laccase B gene was synthesized by McLab Inc. (Molecular Cloning Laboratories, 384 Oyster Point Blvd, Suite 15, South San Francisco, Calif. 94080). The synthetic plasmid DNA was digested with restriction enzymes SpeI and AscI and the 1.5 kb DNA fragment was isolated from gel and cloned into pTrex4 vector which was also digested with SpeI and AscI to create the expression plasmid (pTrex4-laccase-Bopt), which is similar to the expression plasmid shown in FIG. **4** except that the codon optimized laccase B gene replaced the (non-optimized) laccase B gene. The plasmid was transformed biolistically into a *Trichoderma* strain. More than 30 transformants were generated and were transferred to new plates. A total of 20 stable transformants were selected and mycelia were transferred to 30 mls of defined media containing 1 mM copper. The cultures were grown for 4 days at 28° C. Culture broths were centrifuged and supernatants were used for ABTS assay.

Example 11

a. Expression of Laccase D Gene in *Trichoderma*

To construct the expression plasmid for the laccase D gene of the CBS115.075 strain, two primers (SEQ ID No. 63 and SEQ ID No. 64) were used in the Herculase PCR reaction containing genomic DNA template obtained from CBS115.075 strain (see Example 2b). The PCR fragment was purified using the QIAquick spin column and cloned into pENTR/D-TOPO vector. Sixteen clones were amplified using Ready-To-Go PCR beads and four plasmid DNAs were sequenced. The pENTR-laccaseD CBS115.075#2 clone was selected. The pENTR-laccaseD CBS115.075#2 plasmid (50 ng) was converted to expression plasmid pTrex3g-laccaseD, which is similar to the expression plasmid shown in FIG. **1** except that the codon optimized laccase D gene replaced the laccase A gene, in a 10 ul LB clonase II reaction containing

6.5 ul of TE, 1 ul of pTrex3g vector (0.1 mg/ml) and 2 ul of ClonaseII. The expression plasmid was confirmed again by DNA sequencing and transformed biolistically into a *Trichoderma* strain. Forty-five transformants were selected and were transferred to new plates. Mycelia from 28 stable transformants were transferred to 30 mls of defined media containing 0.5 mM copper. The cultures were grown for 4 days at 28° C. Culture broths were centrifuged and supernatants were used for ABTS assay.

b. Expression of the Laccase D Gene in *Trichoderma* as CBH1 Fusion

To construct the expression plasmid for the laccase D gene of the CBS115.075 strain, two primers (GGACTAGTGTCGCCGTTTACAAACGCGCAATTGGGCCCGTGGCCGAC, SEQ ID No. 68) and (AAGGCGCGCCTTAAATAGCAGTTCCTTTCTTAG, SEQ ID No. 69) were designed and obtained from Invitrogen. The primers were used in the Herculase PCR reaction containing genomic DNA of the CBS115.075 strain as the DNA template. The PCR fragment was purified using the QIAquick spin column and digested with restriction enzymes SpeI and AscI and cloned into pTrex4 vector (see U.S. patent application Ser. No. 10/590,956; WO 05/093050) which was also digested with SpeI and AscI to create the expression plasmid (pTrex4-laccaseD). The fidelity of the expression plasmid was confirmed by DNA sequencing and transformed biolistically into *Trichoderma* strain. More than 300 transformants were generated and sixty transformants were transferred to new plates. Mycelia of 25 stable transformants were transferred to 30 mls of defined media containing 0.5 mM copper. The cultures were grown for 4 days at 28° C. Culture broths were centrifuged and supernatants were used for ABTS assay.

Example 12

Expression of the Laccase D Gene in *Trichoderma* as CBH1 Fusion Using Codon Optimized synthetic gene

DNA (SEQ ID NO:70):

```
ACTAGTGTCG CCGTTTACAA ACGCGCTATT GGACCAGTTG CTGATCTGCA     50

CATCGTTAAC AAGGATTTGG CCCCAGACGG CGTCCAGCGC CCAACTGTTC    100

TGGCCGGTGG AACTTTTCCG GGCACGCTGA TTACCGGTCA AAAGGGCGAC    150

AACTTCCAGC TGAACGTGAT TGATGACCTG ACCGACGATC GCATGTTGAC    200

CCCTACTTCG ATCCATTGGC ATGGTTTCTT CCAGAAGGGA ACCGCCTGGG    250

CCGACGGTCC GGCTTTCGTT ACACAGTGCC CTATTATCGC AGACAACTCC    300

TTCCTCTACG ATTTCGACGT TCCCGACCAG GCGGGCACCT TCTGGTACCA    350

CTCACACTTG TCTACACAGT ACTGCGACGG TCTGCGCGGT GCCTTCGTTG    400

TTTACGACCC CAACGACCCT CACAAGGACC TTTATGATGT CGATGACGGT    450

GGCACAGTTA TCACATTGGC TGACTGGTAT CACGTCCTCG CTCAGACCGT    500

TGTCGGAGCT GCTACACCCG ACTCTACGCT GATTAACGGC TTGGGACGCA    550

GCCAGACTGG CCCCGCCGAC GCTGAGCTGG CCGTTATCTC TGTTGAACAC    600

AACAAGAGAT ACCGTTTCAG ACTCGTCTCC ATCTCGTGCG ATCCCAACTT    650

CACTTTTAGC GTCGACGGTC ACAACATGAC GGTTATCGAG GTTGATGGCG    700

TGAATACCCG CCCTCTCACC GTCGATTCCA TTCAAATTTT CGCCGGCCAG    750

CGATACTCCT TTGTGCTGAA TGCCAATCAG CCCGAGGATA ACTACTGGAT    800

CCGCGCTATG CCTAACATCG GACGAAACAC CACTACCCTT GATGGCAAGA    850

ATGCCGCTAT CCTGCGATAC AAGAACGCCA GCGTTGAGGA GCCCAAAACC    900

GTCGGAGGAC CCGCGCAGAG CCCATTGAAC GAGGCCGACC TGCGACCTCT    950

GGTGCCCGCT CCTGTCCCTG GCAACGCAGT TCCTGGTGGT GCGGACATCA   1000

ACCACCGCCT GAACCTGACA TTCAGCAACG GCCTCTTCTC TATCAATAAC   1050

GCATCATTTA CAAACCCCAG CGTCCCTGCC TTGTTGCAGA TTCTTTCCGG   1100

CGCACAAAAC GCTCAGGATC TGCTTCCCAC CGGTTCTTAT ATCGGCTTGG   1150

AGTTGGGCAA GGTCGTTGAA CTCGTGATCC CTCCCTTGGC CGTTGGTGGC   1200

CCCCATCCAT TCCACTTGCA CGGCCACAAC TTTTGGGTCG TCCGAAGCGC   1250

TGGTTCTGAC GAGTATAATT TCGACGATGC AATTTTGCGC GACGTGGTCA   1300

GCATTGGCGC GGGAACTGAC GAGGTTACTA TCCGTTTTGT CACTGATAAC   1350
```

-continued

```
CCAGGCCCTT GGTTCCTCCA TTGCCACATC GACTGGCACC TCGAAGCCGG  1400

CCTCGCCATT GTTTTCGCCG AAGGCATCAA TCAAACCGCA GCCGCCAACC  1450

CGACTCCACA GGCCTGGGAC GAACTCTGCC CCAAGTATAA CGGACTCTCC  1500

GCTTCCCAGA AGTGAAGCC CAAGAAGGGA ACAGCCATCT AAGGCGCGCC  1550
```

encoding the laccase D gene (based on the gene from CBS115.075) was synthesized by DNA2.0 Inc. (1455 Adams Drive, Menlo Park, Calif. 94025). The synthetic plasmid DNA was digested with restriction enzymes SpeI and AscI and The 1.5 kb DNA fragment was isolated from gel and cloned into pTrex4 vector which was also digested with SpeI and AscI to create the expression plasmid (pTrex4-laccase-Dopt). The plasmid was transformed biolistically into a *Trichoderma* strain. Forty transformants were transferred to new plates. A total of 24 stable transformants were selected and mycelia were transferred to 30 mls of defined media containing 0.5 mM copper. The cultures were grown for 4 days at 28° C. Culture broths were centrifuged and supernatants were used for ABTS assay.

Example 13

Expression of the Laccase D Gene in *Bacillus* as BCE103 Fusion Using optimized synthetic gene

DNA (SEQ ID NO:71):

```
GGATCCTGAA GCTATCGGTC CGGTTGCAGA TTTACACATC GTAAACAAAG    50

ATCTTGCACC TGACGGCGTT CAACGTCCAA CTGTACTTGC TGGTGGAACA   100

TTCCCTGGTA CACTTATTAC TGGTCAAAAA GGTGACAACT TCCAATTAAA   150

CGTAATTGAC GATCTTACAG ATGACCGTAT GCTTACACCG ACTTCAATTC   200

ACTGGCACGG TTTCTTTCAA AAAGGAACAG CATGGGCTGA TGGTCCTGCA   250

TTCGTTACAC AATGTCCAAT CATTGCTGAT AACTCTTTCC TTTACGATTT   300

TGACGTTCCT GATCAAGCTG GTACATTCTG GTATCACTCA CACTTATCCA   350

CACAATACTG CGATGGACTT CGCGGAGCTT TCGTAGTTTA CGACCCAAAC   400

GATCCTCATA AAGACCTTTA CGATGTAGAT GATGGTGGAA CAGTTATCAC   450

ATTAGCTGAT TGGTACCATG TACTTGCTCA AACAGTTGTA GGTGCAGCTA   500

CACCAGATTC AACACTTATC AATGGATTAG GACGTTCTCA AACTGGTCCT   550

GCTGACGCAG AACTTGCTGT AATCTCTGTT GAACATAACA AACGTTACAG   600

ATTCCGTCTT GTTAGCATTT CTTGCGATCC AAACTTCACA TTTTCAGTTG   650

ACGGACATAA CATGACAGTT ATCGAAGTAG ATGGTGTAAA CACACGTCCA   700

CTTACTGTAG ACTCTATCCA AATCTTCGCA GGACAACGTT ACTCATTCGT   750

ATTAAACGCA AATCAACCAG AAGATAACTA CTGGATTCGT GCAATGCCAA   800

ACATCGGACG TAACACTACA ACTCTTGACG GCAAAAACGC AGCTATTCTT   850

CGTTACAAAA ACGCTTCTGT TGAAGAACCT AAAACAGTTG GTGGACCAGC   900

ACAATCACCA CTTAACGAAG CTGACTTACG TCCACTGGTT CCAGCACCTG   950

TACCTGGAAA CGCTGTACCA GGAGGTGCTG ATATTAATCA TAGACTTAAC   100

CTTACTTTCT CTAACGGTCT GTTCTCAATC AACAACGCTT CATTCACAAA  1050

TCCTTCAGTT CCAGCACTTT TACAAATTCT TAGCGGTGCA CAAAATGCTC  1100

AGGATCTTTT ACCAACTGGA TCTTACATTG GTCTTGAACT GGGTAAAGTA  1150

GTTGAATTAG TAATTCCTCC GCTTGCTGTA GGTGGACCAC ATCCTTTCCA  1200

TCTTCACGGT CATAACTTCT GGGTTGTACG TTCTGCTGGT TCAGATGAAT  1250

ACAACTTCGA TGACGCAATT CTTCGTGATG TTGTATCTAT TGGTGCTGGA  1300

ACAGATGAAG TAACTATTCG TTTCGTAACA GATAACCCTG GTCCTTGGTT  1350

CTTACATTGT CATATCGATT GGCATCTTGA AGCTGGACTT GCTATTGTTT  1400
```

-continued

```
TCGCTGAAGG AATCAATCAA ACAGCTGCAG CTAACCCAAC ACCTCAAGCA  1450

TGGGACGAAT TATGTCCAAA ATACAACGCA CTTTCTCCAG GAGATACTTA  1500

AAAGCTT                                                 1507
```

encoding the laccase D gene (based on the gene from CBS115.075) was synthesized by DNA2.0 Inc. (1455 Adams Drive, Menlo Park, Calif. 94025). The synthetic plasmid DNA was digested with restriction enzymes BamHI and HindIII and the 1.5 kb DNA fragment was isolated from a gel and ligated into the p2JMagk103lnk2 vector (see US20050202535A1) digested with the same two restriction enzymes to create the expression plasmid p2JMagk103lnk2E-laccase (FIG. **6**). The plasmid was transformed into a *B. subtilis* strain (degUHY32, oppA, DspoIIE, DaprE, DnprE, Depr, DispA, Dbpr, Dvpr, DwprA, DmprybfJ, DnprB, amyE::xylRPxylAcomK-ermC) (see US20050202535A1). Two transformants were selected on Luria Broth agar plates with 5 mg/ml chloramphenicol, and then to select for clones with higher gene copy numbers, colonies were serially streaked on Luria Broth agar plates with 25 mg/ml chloramphenicol until rapid colony growth was obtained. The amplified transformants were inoculated into 30 ml MBD medium (see US20050202535A1) containing 0.5 mM copper. The cultures were grown for 60 h at 37° C. Culture broths were centrifuged and supernatants were used for ABTS assay.

Example 14

Bleaching of Solubilized Indigo with Different Laccases

An assay for the bleaching of the solubilized indigo substrate by laccase/mediator combinations was performed in a 96-well microtitre plate as follows

A saturated solution of indigo in N-methylpyrrolidone (NMP) was prepared by stirring indigo (30 mg) in NMP (10 ml) at room temperature for 5 hours. The NMP solution was diluted 10-fold into an aqueous buffer solution resulting in a blue solution. For example, dilution into 50 mM sodium acetate buffer at pH 5, or 50 mM sodium phosphate buffer at pH 7. Solutions were shaken well immediately before use.

The assay for the bleaching of the solubilized indigo substrate was performed in a 96-well microtitre plate whereby each well received the soluble indigo solution in 50 mM sodium acetate buffer at pH 5 (180 uL), laccase (10 ppm enzyme) and mediator solution (from a 20 mM stock solution in methanol). The total volume of each well was adjusted to 200 uL with deionzed water. A control containing laccase only was run in duplicate. The plate was sealed and incubated at 50° C. for 2 hours at 800 rpm on a heated agitator (Thermomixer, Eppendorf). Following this period, the plates were unsealed and a solution of ascorbic acid (20 uL of a 10% aqueous solution) added to each well in order to reduce the oxidized forms of the mediators. The extent of indigo bleaching was then assessed by determining the absorbance for each well at 600 nm using a microtitre plate reader. The lower the absorbance reading, the greater the extent of indigo bleaching.

FIG. **7** shows the results for a *Thielavia* sp. laccase (Ecostone LCC10, AB enzymes, Darmstadt, Germany). The mediators used were 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS), syringic acid, 4-carboxamido-2,6-dimethoxyphenol (SA), methyl syringate (MS), 4-(N-methyl carboxamido)-2,6-dimethoxyphenol (MSA), 10-(carboxypropyl)-phenothiazine (PTP) and syringaldehyde. The changes in absorbance at 600 nm relative to control are listed in Table 1 where the greatest change in absorbance corresponds to the largest extent of indigo bleaching.

At a mediator concentration of 500 uM, the most effective mediator for indigo bleaching was ABTS, followed by the N-methyl amide (MSA) and the unsubstituted amide, 4-carboxamido-2,6-dimethoxyphenol (SA). At the lower mediator concentration of 50 uM, ABTS was still the most effective mediator, with the remaining mediators being more or less equivalent. The exception was syringic acid, which bleached soluble indigo no more effectively than the control condition.

TABLE 1

Change in absorbance at 600 nm following bleaching of soluble indigo using a *Thielavia* sp. laccase and a variety of mediators at 500 and 50 uM concentrations (n = 2).

	500 mM Concentration		50 mM Concentration	
Mediator	ΔA600	Std Dev	ΔA600	Std Dev
Control	0	0.008	0	0.010
ABTS	0.235	0.019	0.174	0.032
Syringic acid	0.024	0.017	0.005	0.009
SA	0.170	0.018	0.088	0.014
Methyl Syringate	0.062	0.035	0.090	0.012
MSA	0.181	0.013	0.103	0.018
PTP	0.044	0.009	0.132	0.020
Syringaldehyde	0.132	0.012	0.092	0.017

Example 15

Soluble Indigo Bleaching Assay with Different Laccases at Two pH Values

Laccases derived from *Myceliophtora* (Denilite® II, Novozymes, Bagsvaerd, Denmark), *Thielavia* (Ecostone LCC10, AB enzymes, Darmstadt, Germany) and *Cerrena* sp. were assessed for their ability to bleach solubilized indigo in conjunction with low molecular weight mediators at two pH values.

Bleaching of solubilized indigo in 96-well microtitre plates was performed as described in Example 14, using 3 different laccases at pH values of 5 and 7. The mediators used were sinapinic acid, 4-carboxamido-2,6-dimethoxyphenol (SA), methyl 4-acetyl syringate (AMS), methyl syringate (MS) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS). FIGS. **8** and **9** shows the results of soluble indigo bleaching at pH values of 5 and 7 using three laccases derived from Myceliophtora, *Thielavia* and *Cerrena* sp. respectively. These data are tabulated in Tables 2 and 3.

TABLE 2

Change in absorbance at 600 nm relative to a control following bleaching of soluble indigo using laccases from *Thielavia*, *Myceliophtora* and *Cerrena* sp. at pH 5, at a mediator concentration of 250 uM.

	Laccase					
	Thielavia		*Myceliophtora*		*Cerrena*	
Mediator	ΔA_{600}	Std Dev	ΔA_{600}	Std Dev	ΔA_{600}	Std Dev
Control 1	0	0.016	0	0.010	0	0.005
Sinapinic acid	0.068	0.019	0.157	0.020	0.240	0.007
SA	0.170	0.011	0.254	0.013	0.142	0.005
AMS	0.100	0.012	0.117	0.007	0.028	0.003
MS (AB)	0.048	0.011	0.057	0.007	0.005	0.011
MS (Denilite)	0.050	0.013	0.061	0.007	0.043	0.013
ABTS	0.234	0.012	0.267	0.008	0.329	0.031
Control 2	−0.007	0.017	−0.011	0.007	−0.006	0.005

TABLE 3

Change in absorbance at 600 nm relative to a control following bleaching of soluble indigo using laccases from *Thielavia*, *Myceliophtora* and *Cerrena* sp. at pH 7, at a mediator concentration of 250 uM.

	Laccase					
	Thielavia		*Myceliophtora*		*Cerrena*	
Mediator	ΔA600	Std Dev	ΔA600	Std Dev	ΔA600	Std Dev
Control 1	0	0.008	0	0.001	0	0.006
Sinapinic acid	0.112	0.015	0.204	0.020	0.257	0.005
SA	0.162	0.006	0.220	0.009	0.128	0.010
AMS	0.087	0.006	0.078	0.005	0.077	0.007
MS (AB)	0.053	0.010	0.076	0.006	0.000	0.006
MS (Denilite)	0.069	0.017	0.086	0.001	0.008	0.018
ABTS	0.145	0.006	0.155	0.014	0.215	0.056
Control 2	0.007	0.006	−0.004	0.001	0	0.005

Example 16

Purification and Determination of Specific Activity

The laccase D optimized gene (SEQ ID NO:70) was expressed using the expression system described in co-pending application U.S. 60/984,430 (Attorney Docket No. GC993P entitled "Signal Sequences and co-expressed chaperones for improved heterologous protein production in a host cell" filed 1 Nov. 2007) in 14 liter fermenters. Fermentation broth from was harvested at 184 hours and concentrated by ultra filtration (UFC 20070245). The concentrate was diafiltered into 25 mM sodium acetate, pH4.0 buffer. Then 500 ml of the diafiltered UFC sample was loaded on to an ion exchange column containing Poros HS-20 resin (Applied Biosystems, 20×275 mm column) equilibrated with 25 mM sodium acetate buffer, pH 4.0. The column was washed with 10 column volumes of 25 mM sodium acetate buffer, pH 4.0. The laccase D protein was eluted from the column using a salt gradient (12 column volumes) from 40 mm to 80 mM sodium chloride in 25 mM sodium acetate buffer, pH 4.0. Fractions containing laccase activity were pooled and further concentrated using an Amicon 400 mL stir cell with a 10K membrane. Total protein was measure by SDS protein gel using BSA as standard as 4 mg/ml (>90% pure). The laccase sample was diluted 10,000 fold with water and stored at RT for 18 hours and at 4° C. for more than 24 hours. ABTS activity was measured as 8570 units/ml. The specific activity of the recombinant laccase D is then calculated by dividing 8570 units/ml by 4 mg/ml resulting in 2140 units/mg of protein which is 100 times more activity than the Stachybotrys laccase (16 u/mg), see Mander et al, Appl. Environ. Microbiol. (2006) 72:5020-5026). Thus, this enzyme results in lower copper discharge into the environment than other laccases, e.g., Stachybotrys laccase, by virtue of the high specific activity.

Example 17

Procedure for Denim Bleaching

Mediators

4-hydroxy-3,5-dimethoxybenzamide (syringamide, SA) was purchased from Punjab Chemicals & Crop Protection Limited (Mumbai, India). 4-hydroxy-3,5-dimethoxybenzonitrile (syringonitrile, SN) was acquired from StereoChemical, Inc., (Newark, Del.) or Punjab Chemicals & Crop Protection Limited (Mumbai, India).

Enzyme

Laccase enzyme, derived from *Cerrena unicolor* (Example 16, 8570 U/ml, 4 mg protein/ml) was used in the experiments.

Procedure

The enzyme incubations were done in an ATLAS LP 2 Launder-O-meter at different conditions in relation to pH, temperature, enzyme concentration and mediator concentration.

Reactions were carried out in 500 ml stainless steel reaction vessels containing 100 ml of liquid. To each vessel five (7×7 cm) stonewashed denim swatches (ACG denim style 80270) and 6 steel balls of 6 mm diameter were added. The reactions vessels were closed and entered into the launder-O-meter that was pre-heated to the desired temperature. The incubation was carried out for 30 minutes after which the swatches were washed with 'running' tap water, spin dried in an AEG IPX4 centrifuge and dried with an Elna Press Electronic iron at program cotton and evaluated.

Stonewashing of Denim

Denim, 12 legs weighing approximately 3 kg, was desized in a Unimac UF 50 washing machine under the following conditions:

Desizing for 15 minutes at 10:1 liquor ratio 50° C. with 0.5 g/l (15 g) of Optisize 160 amylase (Genencor) and 0.5 g/l (15 g) of a non-ionic surfactant (e.g. Rucogen BFA, (Rudolf Chemie) or Ultravon GPN, (Huntsman))

2 cold rinses for 5 minutes at 30:1 liquor ratio.

Following desizing the denim was stonewashed in a Unimac UF 50 washing machine under the following conditions:

Cold rinse for 5 minutes at 10:1 liquor ratio

Stonewashing for 60 minutes at 10:1 liquor ratio 55° C. with 1 kg of pumice stone, citrate buffer (30 g tri-sodium citrate dihydrate and 30 g citric acid monohydrate) and 35 g IndiAge 2XL cellulase (Genencor).

2 cold rinses for 5 minutes at 30:1 liquor ratio.

The denim was dried in a Miele Novotronic T494C household fabric dryer. From the denim legs, swatches of 7×7 cm were cut.

Evaluation of Denim Swatches

The color of the five denim swatches is measured with a Minolta Chromameter CR 310 in the CIE Lab color space with a D 65 light source. Measurements were done before and after laccase treatment and the results of the five swatches were averaged. The total color difference (TCD) is calculated. The total color difference can be calculated with the formula: $TCD=\sqrt{(\Delta L)^2+(\Delta a)^2+(\Delta b)^2}$.

Evaluation of Denim Legs

Denim legs were evaluated with a Minolta Chromameter CR 310 in the CIE Lab color space with a D 65 light source. Measurements were done only after laccase treatment. For each denim leg 8 measurements are taken and the result of the 12 legs (96 measurements) was averaged. The total color difference (ΔE) is calculated from the difference between the initial and final CIE L*a*b* values according to the formula

$$\Delta E=(\Delta L^2+\Delta a^2+\Delta b^2)^{1/2}$$

Example 18

Effect of Temperature on the Recombiant Laccase D Bleaching Performance (Unimac)

Laccase bleaching of stonewashed denim: Denim, 12 legs approximately 3 kg, was desized and stonewashed as described in example 17. After stonewashing a laccase treatment was done in a Unimac UF 50 washing machine according to the following process:

30 minutes at 10:1 liquor ratio,

pH 6 (21 g monosodium phosphate and 5 g adipic acid, laccase D laccase) or pH 4.8 (8.6 g monosodium phosphate and 16.8 g of adipic acid, Novoprime Base 268 laccase)

laccase (laccase D or Novoprime Base 268)

mediator (syringamide (SA) and syringonitrile (SN))

After laccase treatment the denim use rinsed twice in cold water for 5 minutes at 30:1 liquor ratio.

The laccase experiments were carried out and the results are presented in Tables 4 and 5.

TABLE 4

Laccase D concentration	Mediator	Mediator concentration	Temperature (° C.)	Bleaching level (CIE L)
0.05 g/l/0.4 U/ml	SA	0.33 mM	60	35.6
0.05 g/l/0.4 U/ml	SN	0.47 mM	60	35.9
0.05 g/l/0.4 U/ml	SA	0.33 mM	40	35.6
0.05 g/l/0.4 U/ml	SN	0.47 mM	40	35.7

TABLE 5

Novoprime base 268 concentration	Mediator concentration	Temperature (° C.)	Bleaching level (CIE L)
0.05 g/l	0.023 g/l	60	35.9
0.05 g/l	0.023 g/l	40	33.7

The recombinant laccase D has better performance at lower temperatures than currently available commercial laccases. The laccase D (in the presence of mediator) provides a bleaching effect at temperatures below 60° C., preferably between 40° C. and 60° C. Thus, the laccase may provide an energy benefit to the textile processor.

Example 19

Effect of Recombinant Laccase Enzyme and Mediator Concentration on Bleaching Performance (Launder-O-Meter)

The effect of laccase and mediator concentration was evaluated running the experiments in the table below at pH 6 (50 mM monosodium phosphate buffer pH adjusted with sodium hydroxide 4N solution) and a temperature of 60° C.

The experiments were done with syringamide (SA)— and syringonitrile (SN) mediator.

100 ml buffer was added to a beaker with five swatches, 7×7 cm. The total weight 12 g, (denim:liquor ratio=1:8). Laccase and mediator concentrations were used as indicated in the tables below.

TABLE 6

Laccase enzyme concentration (μl/l)	Activity correspondence (Laccase unit/g denim)
10	0.67
33	2.17
55	3.67
78	5.17
100	6.67

TABLE 7

Mediator Concentration (mM)
0.10
0.33
0.55
0.78
1.00

The amounts of syringamide or syringonitrile mediator as indicated in the tables below were added to each beaker as a dilution of a 275 mM SA— or —SN stock solution in 98% methanol. The laccase was added to each beaker as indicated in the tables below, as dilution of a 400 units/ml laccase stock solution. The beakers were closed and processed at 60° C. as described in the example 17. The swatches were evaluated as described in example 17.

TABLE 8

LACCASE + SA at 60° C. pH 6

Laccase (μl/l)	Mediator syringamide (mM)	TCD
100	1.00	5.6
100	1.00	6.0
100	0.10	2.9
78	0.33	4.4
55	1.00	6.2
55	0.55	5.3
33	0.78	5.5
33	0.33	4.6
10	1.00	3.2
10	0.10	2.5
55	0.55	5.8
100	0.55	5.3
78	0.78	5.9
100	0.10	3.2
55	0.10	3.1
10	0.55	3.6

TCD = total color difference

TABLE 9

LACCASE + SN at 60° C. pH 6

Laccase (μl/l)	Mediator syringonitrile (mM)	TCD
100	1.00	7.6
100	1.00	8.1
100	0.10	4.1

TABLE 9-continued

LACCASE + SN at 60° C. pH 6		
Laccase (µl/l)	Mediator syringonitrile (mM)	TCD
78	0.33	5.6
55	1.00	7.0
55	0.55	6.0
33	0.78	5.5
33	0.33	4.4
10	1.00	3.8
10	0.10	2.7
55	0.55	6.3
100	0.55	7.1
78	0.78	7.1
100	0.10	4.0
55	0.10	3.5
10	0.55	3.4

TCD = total color difference

The above Tables and FIGS. **10** and **11** show that you need both enzyme and mediator to get bleaching. Also it shows there is some flexibility in the enzyme/mediator ratio in achieving a certain bleaching level.

Example 20

Recombinant Laccase D Dose Response Effect on the Bleaching Performance (Unimac)

Laccase bleaching of stonewashed denim—Denim, 12 legs weighing approximately 3 kg, was desized and stonewashed as described in Example 17. After stonewashing, a laccase treatment was done according to the following process: 30 minutes at 10:1 liquor ratio and pH 6 (21 g monosodium phosphate and 5 g adipic acid) and 60° C. with laccase and mediator. After laccase treatment the denim use rinsed twice in cold water for 5 minutes at 30:1 liquor ratio.

The following experiments were carried out.

Syringamide 0.33 mM:

Cerrena unicolor laccase concentration (g/l)	Bleaching level (CIE L)
0.010	34.6
0.05	36.2
0.25	36.2

Syringonitrile 0.39 mM:

Laccase D concentration (g/l)	Bleaching level (CIE L)
0.25	37.7
0.4	39.5
0.53	38.8

The results are shown in the above tables. This shows that with recombinant laccase D and the amide mediator the bleaching level flattens quite quickly. With an enzyme concentration of 0.05 and 0.25 the same bleaching level is obtained. For the recombinant laccase D and the nitrile mediator the bleaching level increases up to 0.4 g/l, where there appears to be an optimum.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

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Phe Val Val Tyr Asp Pro Lys Asp Pro Asn Lys Arg Leu Tyr Asp Ile
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Pro Leu Leu Glu Gln Asn Leu Arg Pro Leu Val Tyr Thr Pro Val Pro
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Gly Asn Pro Thr Pro Gly Gly Ala Asp Ile Val His Thr Leu Asp Leu
            340                 345                 350

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        355                 360                 365

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Phe His Leu His Gly His Asn Phe Trp Val Val Arg Ser Ala Gly Thr
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Gln Thr Ser Ile His Trp His Gly Phe Phe Gln Lys Gly Ser Ala Trp
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Ala Asp Gly Pro Ala Phe Val Thr Gln Cys Pro Ile Val Thr Gly Asn
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Phe Val Val Tyr Asp Pro Lys Asp Pro Asn Lys Arg Leu Tyr Asp Ile
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Ala Arg Thr Val Val Gly Val Ala Thr Pro Asp Ala Thr Leu Ile Asn
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Gly Leu Gly Arg Ser Pro Asp Gly Pro Ala Asp Ala Glu Leu Ala Val
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Ile Asn Val Lys Arg Gly Lys Arg Tyr Arg Phe Arg Leu Val Ser Ile
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Ser Cys Asp Pro Asn Tyr Ile Phe Ser Ile Asp Asn His Ser Met Thr
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Val Ile Glu Val Asp Gly Val Asn Thr Gln Ser Leu Thr Val Asp Ser
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385                 390                 395                 400

Val Val Glu Leu Val Ile Pro Ala Gly Val Val Gly Gly Pro His Pro
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Phe His Leu His Gly His Asn Phe Trp Val Val Arg Ser Ala Gly Thr
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Thr Phe Pro Gly Pro Leu Ile Arg Gly Asn Lys Gly Asp Asn Phe Lys
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Ile Asn Val Ile Asp Asp Leu Thr Glu His Ser Met Leu Lys Ala Thr
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Ser Ile His Trp His Gly Phe Phe Gln Lys Gly Thr Asn Trp Ala Asp
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Gly Pro Ala Phe Val Thr Gln Cys Pro Ile Thr Ser Gly Asn Ala Phe
100 105 110

Leu Tyr Asp Phe Asn Val Pro Asp Gln Ala Gly Thr Phe Trp Tyr His
115 120 125

Ser His Leu Ser Thr Gln Tyr Cys Asp Gly Leu Arg Gly Ala Phe Val
130 135 140

Val Tyr Asp Pro Asn Asp Pro Asn Lys Gln Leu Tyr Asp Val Asp Asn
145 150 155 160

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Gly Asn Thr Val Ile Thr Leu Ala Asp Trp Tyr His Ala Leu Ala Gln
                165                 170                 175

Thr Val Thr Gly Val Ala Val Ser Asp Ala Thr Leu Ile Asn Gly Leu
            180                 185                 190

Gly Arg Ser Ala Thr Gly Pro Ala Asn Ala Pro Leu Ala Val Ile Ser
        195                 200                 205

Val Glu Arg Asn Lys Arg Tyr Arg Phe Arg Leu Val Ser Ile Ser Cys
    210                 215                 220

Asp Pro Asn Phe Ile Phe Ser Ile Asp His His Pro Met Thr Val Ile
225                 230                 235                 240

Glu Met Asp Gly Val Asn Thr Gln Ser Met Thr Val Asp Ser Ile Gln
                245                 250                 255

Ile Phe Ala Gly Gln Arg Tyr Ser Phe Val Met Gln Ala Asn Gln Pro
            260                 265                 270

Val Gly Asn Tyr Trp Ile Arg Ala Lys Pro Asn Val Gly Asn Thr Thr
        275                 280                 285

Phe Leu Gly Gly Leu Asn Ser Ala Ile Leu Arg Tyr Val Gly Ala Pro
    290                 295                 300

Asp Gln Glu Pro Thr Thr Asp Gln Thr Pro Asn Ser Thr Pro Leu Val
305                 310                 315                 320

Glu Ala Asn Leu Arg Pro Leu Val Tyr Thr Pro Val Pro Gly Gln Pro
                325                 330                 335

Phe Pro Gly Gly Ala Asp Ile Val Lys Asn Leu Ala Leu Gly Phe Asn
            340                 345                 350

Ala Gly Arg Phe Thr Ile Asn Gly Ala Ser Leu Thr Pro Pro Thr Val
        355                 360                 365

Pro Val Leu Leu Gln Ile Leu Ser Gly Thr His Asn Ala Gln Asp Leu
    370                 375                 380

Leu Pro Ala Gly Ser Val Ile Glu Leu Glu Gln Asn Lys Val Val Glu
385                 390                 395                 400

Ile Val Leu Pro Ala Ala Gly Ala Val Gly Gly Pro His Pro Phe His
                405                 410                 415

Leu His Gly His Asn Phe Trp Val Val Arg Ser Ala Gly Gln Thr Thr
            420                 425                 430

Tyr Asn Phe Asn Asp Ala Pro Ile Arg Asp Val Val Ser Ile Gly Gly
        435                 440                 445

Ala Asn Asp Gln Val Thr Ile Arg Phe Val Thr Asp Asn Pro Gly Pro
    450                 455                 460

Trp Phe Leu His Cys His Ile Asp Trp His Leu Glu Ala Gly Phe Ala
465                 470                 475                 480

Val Val Phe Ala Glu Gly Ile Asn Gly Thr Ala Ala Ala Asn Pro Val
                485                 490                 495

Pro Ala Ala Trp Asn Gln Leu Cys Pro Leu Tyr Asp Ala Leu Ser Pro
            500                 505                 510

Gly Asp Thr
        515
```

<210> SEQ ID NO 7
<211> LENGTH: 2663
<212> TYPE: DNA
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 7

caccgcgatg tctcttcttc gtagcttgac ctccctcatc gtactagcca ctggtgcatt 60

tgctgcaatc ggtccagtca ccgacctaca tatagtgaac cagaatctcg ccccagatgg 120

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tttaaaccgc cccactgtac tcgcaggtgg tactttcccc ggtcctctga ttcgtggtaa 180

caaggtacgc ttcataaccg ccctccgtag acgtaggctt cggctgacat gaccatcatc 240

tgtagggaga taactttaaa attaatgtga ttgacgactt gacagaacac agtatgctca 300

aggctacgtc cattgtaagt ccctgattaa cgtttcacct ggtcatatcg ctcaacgtct 360

cgaagcactg gcatgggttc ttccagaagg gaaccaactg ggccgatggc cccgcctttg 420

tcacccaatg tcctatcaca tcaggaaacg ccttcttgta tgatttcaac gttccggacc 480

aagctggtac tttctggtac cacagccatc tctcyacaca gtattgtgac ggtcttcgtg 540

gtgcctttgt cgtctatgat cctaatgatc ccaacaagca actctatgat gttgataacg 600

gcaagtccct tgcatatttc agttctatca tatcctcacc tgtattggca cagaaagcac 660

cgtgattacc ttggctgatt ggtatcatgc ccttgctcag actgtcactg gtgtcgcgtg 720

agtgacaaat ggcccttaat tgttcacata ttttcctgat tatcatatga tagagtatct 780

gatgcaacgt tgatcaacgg attgggacgt tcggccaccg gccccgcaaa tgcccctctg 840

gcggtcatca gtgtcgagcg gaataagagg tcagttccat aattatgatt atttcccgcg 900

ttacttccta acgattattt ttgtatccct ccacagatat cgtttccgat tggtttctat 960

ttcttgcgac cctaacttta ttttctcaat tgaccaccac ccaatgaccg taattgagat 1020

ggacggtgtt aatacccaat ctatgaccgt agattcgatc caaatattcg caggtcaacg 1080

atattcattt gtcgtaggtt attataaact gcccaccgat catctctcac gtaactgtta 1140

tagatgcaag ccaaccaacc agttggaaat tattggatcc gygctaaacc taatgttggg 1200

aacacaactt tccttggagg cctgaactcc gctatattac gatatgtggg agcccctgac 1260

caagaaccga ccactgacca aacacccaac tctacaccgc tcgtcgaggc gaacctacgt 1320

cccctcgtct atactcctgt ggtatgttgt tctcgttaca tataccaaac ctaatatgag 1380

gactgaacgg atctactagc cgggacagcc attccctggc ggtgctgata tcgtcaagaa 1440

cttagctttg ggtttcgtac gtgtatttca cttccctttt ggcagtaact gaggtggaat 1500

gtatatagaa tgccgggcgt ttcacaatca atggaacatc cttcacacct cctacagtcc 1560

ctgtactact ccagatcctc agtggtactc acaatgcaca ggatcttctt ccagcaggaa 1620

gcgtgatcga acttgaacag aataaagttg tcgaaatcgt tctgcccgct gcgggcgccg 1680

ttggcggtcc tcatcctttc cacttacatg gtgtaagtat cagacgtcct catgcctata 1740

ttgctccgaa ccttacacac ctgatttcag cacaatttct gggtggttcg tagcgccggt 1800

caaaccacat acaatttcaa tgatgctcct atccgtgatg ttgtcagtat tggcggtgca 1860

aacgatcaag tcacgatccg atttgtggta tgtatctcgt gccttgcatt cattccacga 1920

gtaatgatcc ttacacttcg ggttctcaga ccgataaccc tggcccatgg ttccttcact 1980

gtcacattga ctggcatttg gaggctgggt tcgctgtagt ctttgcggag ggaatcaatg 2040

gcactgcagc tgctaatcca gtcccaggta agactctcgc tgctttgcgt aatatctatg 2100

aatttaaagc atatcaattt gcagcggctt ggaatcaatt gtgcccgttg tatgatgcct 2160

tgagcccagg tgatacatga ttactcgtag ctgtgctttc ttatacatat tctatgggta 2220

tatcggagta gctgtactat agtatgtact atactaggtg ggatatgytg atgttgattt 2280

atataatttt gtttgaagag tgactttatc gacttgggat ttagccgagt acatactgat 2340

ctctcactac aggcttgttt tgtctttggg cgcttactca acagttgact gtttttgcta 2400

ttacgcattg aaccgcattc cggtcygact cgtgtcctct actgtgactt gtattggcat 2460

tctagcacat atgtctctta cctataggaa caatatgtct caacactgtt ccaaaacctg 2520

-continued

cgtaaaccaa atatcgtcca tcagatcaga tcattaacag tgccgcacta acctaataca 2580

ctggcargga ctgtggaaat ccctataaat gacctctaga ccgtgaggtc attgcaaggt 2640

cgctctcctt gtcaagatga ccc 2663

<210> SEQ ID NO 8
<211> LENGTH: 515
<212> TYPE: PRT
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 8

Met Ser Leu Leu Arg Ser Leu Thr Ser Leu Ile Val Leu Ala Thr Gly
1 5 10 15

Ala Phe Ala Ala Ile Gly Pro Val Thr Asp Leu His Ile Val Asn Gln
20 25 30

Asn Leu Ala Pro Asp Gly Leu Asn Arg Pro Thr Val Leu Ala Gly Gly
35 40 45

Thr Phe Pro Gly Pro Leu Ile Arg Gly Asn Lys Gly Asp Asn Phe Lys
50 55 60

Ile Asn Val Ile Asp Asp Leu Thr Glu His Ser Met Leu Lys Ala Thr
65 70 75 80

Ser Ile His Trp His Gly Phe Phe Gln Lys Gly Thr Asn Trp Ala Asp
85 90 95

Gly Pro Ala Phe Val Thr Gln Cys Pro Ile Thr Ser Gly Asn Ala Phe
100 105 110

Leu Tyr Asp Phe Asn Val Pro Asp Gln Ala Gly Thr Phe Trp Tyr His
115 120 125

Ser His Leu Ser Thr Gln Tyr Cys Asp Gly Leu Arg Gly Ala Phe Val
130 135 140

Val Tyr Asp Pro Asn Asp Pro Asn Lys Gln Leu Tyr Asp Val Asp Asn
145 150 155 160

Gly Asn Thr Val Ile Thr Leu Ala Asp Trp Tyr His Ala Leu Ala Gln
165 170 175

Thr Val Thr Gly Val Ala Val Ser Asp Ala Thr Leu Ile Asn Gly Leu
180 185 190

Gly Arg Ser Ala Thr Gly Pro Ala Asn Ala Pro Leu Ala Val Ile Ser
195 200 205

Val Glu Arg Asn Lys Arg Tyr Arg Phe Arg Leu Val Ser Ile Ser Cys
210 215 220

Asp Pro Asn Phe Ile Phe Ser Ile Asp His His Pro Met Thr Val Ile
225 230 235 240

Glu Met Asp Gly Val Asn Thr Gln Ser Met Thr Val Asp Ser Ile Gln
245 250 255

Ile Phe Ala Gly Gln Arg Tyr Ser Phe Val Met Gln Ala Asn Gln Pro
260 265 270

Val Gly Asn Tyr Trp Ile Arg Ala Lys Pro Asn Val Gly Asn Thr Thr
275 280 285

Phe Leu Gly Gly Leu Asn Ser Ala Ile Leu Arg Tyr Val Gly Ala Pro
290 295 300

Asp Gln Glu Pro Thr Thr Asp Gln Thr Pro Asn Ser Thr Pro Leu Val
305 310 315 320

Glu Ala Asn Leu Arg Pro Leu Val Tyr Thr Pro Val Pro Gly Gln Pro
325 330 335

Phe Pro Gly Gly Ala Asp Ile Val Lys Asn Leu Ala Leu Gly Phe Asn
340 345 350

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Ala Gly Arg Phe Thr Ile Asn Gly Thr Ser Phe Thr Pro Pro Thr Val
        355                 360                 365

Pro Val Leu Leu Gln Ile Leu Ser Gly Thr His Asn Ala Gln Asp Leu
    370                 375                 380

Leu Pro Ala Gly Ser Val Ile Glu Leu Glu Gln Asn Lys Val Val Glu
385                 390                 395                 400

Ile Val Leu Pro Ala Ala Gly Ala Val Gly Gly Pro His Pro Phe His
                405                 410                 415

Leu His Gly His Asn Phe Trp Val Val Arg Ser Ala Gly Gln Thr Thr
            420                 425                 430

Tyr Asn Phe Asn Asp Ala Pro Ile Arg Asp Val Val Ser Ile Gly Gly
        435                 440                 445

Ala Asn Asp Gln Val Thr Ile Arg Phe Val Thr Asp Asn Pro Gly Pro
    450                 455                 460

Trp Phe Leu His Cys His Ile Asp Trp His Leu Glu Ala Gly Phe Ala
465                 470                 475                 480

Val Val Phe Ala Glu Gly Ile Asn Gly Thr Ala Ala Ala Asn Pro Val
                485                 490                 495

Pro Ala Ala Trp Asn Gln Leu Cys Pro Leu Tyr Asp Ala Leu Ser Pro
            500                 505                 510

Gly Asp Thr
        515
```

<210> SEQ ID NO 9
<211> LENGTH: 1337
<212> TYPE: DNA
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 9

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gtgggggcgg atccctaact gtttcgaatc ggcaccgaag tatgcaggtg tgacggagat     60

gaggcgtttt ttcatcttcc actgcagtat aaaatgtctc aggtaacgtc cagtttttg    120

taccagagct acctccaaat acctttactc gcaaaggttt cgcgatgtct cttcttcgta    180

gcttgacctc cctcatcgta ctagccactg gtgcatttgc tgcaatcggt ccagtcactg    240

acctacatat agtgaaccag aatctcgccc cagatggttt caaccgcccc actgtactcg    300

caggtggtac tttccccggt cctctgattc gtggtaacaa ggtacgcttc ataaccgccc    360

tccgtagacg taggcttcgg ctgacatgac catcatctgt agggagataa ctttaaaatt    420

aatgtgattg acgacttgac agaacacagt atgctcaagg ccacgtccat tgtaagtccc    480

tgattaacgt ttcacctggt catatcgctc aacgtctcga agcactggca tgggttcttc    540

cagaagggaa ccaactgggc cgatggcccc gcctttgtca cccaatgtcc tatcacatca    600

ggaaactcct tcctgtatga tttcaacgtt ccggaccaag ctggtacttt ctggtaccac    660

agccatctct ctacacagta ttgtgacggt cttcgtggtg cctttgtcgt ctatgatcct    720

aatgatccca acaagcaact ctatgatgtt gataacggca agtcccttgc atatttcatt    780

tctatcatat cctcacctgt attggcacag aaagcaccgt gattaccttg gctgattggt    840

atcatgccct tgctcagact gtcactggtg tcgcgtgagt gacaaatggc cctcaattgt    900

tcacatattt tcctgattat catatgatag agtatctgat gcaacgttga tcaacggatt    960

gggacgttcg gccaccggcc ccgcaaatgc ccctctggcg gtcatcagtg tcgagcggaa   1020

taagaggtca gttccataat tatgattatt tcccgcgtta cttcctaaca attattcttg   1080

tatccctcca cagatatcgc ttccgattgg tgtctatttc ttgcgaccct aactttattt   1140
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tctcaattga tcaccaccca atgaccgtaa ttgagatgga cggtgttaat acccaatcta 1200

tgaccgtaga ttcgatccaa atattcgcag gtcaacgata ttcatttgtc gtaggttatt 1260

ataaactgcc caccgatcat ctctcacgta actgttatag atgcaagcca accaaccrgt 1320

tggaaattat tggatcc 1337

<210> SEQ ID NO 10
<211> LENGTH: 278
<212> TYPE: PRT
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 10

Met Ser Leu Leu Arg Ser Leu Thr Ser Leu Ile Val Leu Ala Thr Gly
1 5 10 15

Ala Phe Ala Ala Ile Gly Pro Val Thr Asp Leu His Ile Val Asn Gln
20 25 30

Asn Leu Ala Pro Asp Gly Phe Asn Arg Pro Thr Val Leu Ala Gly Gly
35 40 45

Thr Phe Pro Gly Pro Leu Ile Arg Gly Asn Lys Gly Asp Asn Phe Lys
50 55 60

Ile Asn Val Ile Asp Asp Leu Thr Glu His Ser Met Leu Lys Ala Thr
65 70 75 80

Ser Ile His Trp His Gly Phe Phe Gln Lys Gly Thr Asn Trp Ala Asp
85 90 95

Gly Pro Ala Phe Val Thr Gln Cys Pro Ile Thr Ser Gly Asn Ser Phe
100 105 110

Leu Tyr Asp Phe Asn Val Pro Asp Gln Ala Gly Thr Phe Trp Tyr His
115 120 125

Ser His Leu Ser Thr Gln Tyr Cys Asp Gly Leu Arg Gly Ala Phe Val
130 135 140

Val Tyr Asp Pro Asn Asp Pro Asn Lys Gln Leu Tyr Asp Val Asp Asn
145 150 155 160

Gly Lys Thr Val Ile Thr Leu Ala Asp Trp Tyr His Ala Leu Ala Gln
165 170 175

Thr Val Thr Gly Val Ala Val Ser Asp Ala Thr Leu Ile Asn Gly Leu
180 185 190

Gly Arg Ser Ala Thr Gly Pro Ala Asn Ala Pro Leu Ala Val Ile Ser
195 200 205

Val Glu Arg Asn Lys Arg Tyr Arg Phe Arg Leu Val Ser Ile Ser Cys
210 215 220

Asp Pro Asn Phe Ile Phe Ser Ile Asp His His Pro Met Thr Val Ile
225 230 235 240

Glu Met Asp Gly Val Asn Thr Gln Ser Met Thr Val Asp Ser Ile Gln
245 250 255

Ile Phe Ala Gly Gln Arg Tyr Ser Phe Val Met Gln Ala Asn Gln Pro
260 265 270

Val Gly Asn Tyr Trp Ile
275

<210> SEQ ID NO 11
<211> LENGTH: 1080
<212> TYPE: DNA
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 11

tgcaatcgga ccggtbgctg accttcacat tacggacgat accattgccc ccgatggttt 60

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ctctcgtcct gctgttctcg ctggcggggg tttccctggc cctctcatca ccggaaacaa 120

ggtaatgcct aatggttgcg tctttgttgg tgctctcatt catccacgac attttgtacc 180

agggcgacgc ctttaaactc aatgtcatcg atgaactaac ggacgcatcc atgctgaagy 240

cgacttccat cgtaagtctc gctgtattgc tccttgagcc atttcattga ctataactac 300

aaccagcact ggcatggatt cttccaaaag ggtactaatt gggcagatgg tcccgctttt 360

gtgaaccaat gccccatcac cacgggaaac tccttcttgt acgacttcca ggttcctgat 420

caagctggta agcatgagat tacactagga aagtttaatt taataactat tcaatcagga 480

acctactggt atcatagtca tttgtctacg caatactgtg atggtctcag aggtgcattc 540

gttgtctacg acccttcaga tcctcacaag gatctctacg acgtcgacga cggtgagctt 600

tgctttttc attggtatcc attatcgctc acgtgtcatt actgcgccac agaaagtacc 660

gtcatcactt tggctgattg gtatcatact ttggctcgtc agattgttgg cgttgcgtga 720

gtagtcttgt accgactgaa acatattcca gttgctgact tccccacagc atttctgata 780

ctaccttgat aaacggtttg ggccgcaata ccaatggtcc ggctgatgct gctcttgctg 840

tgatcaatgt tgacgctggc aaacggtgtg tccagattac tatactcccc atgacgtctc 900

aatgctgatg tgtactactt ccaggtaccg tttccgtctt gtttccatat cctgtgaccc 960

caattgggta ttctcgattg acaaccatga ctttacggtc attgaagtcg atggtgttaa 1020

cagtcaacct ctcaacgtcg attctgttca gatcttcgcc ggacaacgtt actcgttcgt 1080

<210> SEQ ID NO 12
<211> LENGTH: 246
<212> TYPE: PRT
<213> ORGANISM: Cerrena unicolor
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60)..(60)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 12

Ala Ile Gly Pro Val Ala Asp Leu His Ile Thr Asp Asp Thr Ile Ala
1 5 10 15

Pro Asp Gly Phe Ser Arg Pro Ala Val Leu Ala Gly Gly Gly Phe Pro
20 25 30

Gly Pro Leu Ile Thr Gly Asn Lys Gly Asp Ala Phe Lys Leu Asn Val
35 40 45

Ile Asp Glu Leu Thr Asp Ala Ser Met Leu Lys Xaa Thr Ser Ile His
50 55 60

Trp His Gly Phe Phe Gln Lys Gly Thr Asn Trp Ala Asp Gly Pro Ala
65 70 75 80

Phe Val Asn Gln Cys Pro Ile Thr Thr Gly Asn Ser Phe Leu Tyr Asp
85 90 95

Phe Gln Val Pro Asp Gln Ala Gly Thr Tyr Trp Tyr His Ser His Leu
100 105 110

Ser Thr Gln Tyr Cys Asp Gly Leu Arg Gly Ala Phe Val Val Tyr Asp
115 120 125

Pro Ser Asp Pro His Lys Asp Leu Tyr Asp Val Asp Asp Glu Ser Thr
130 135 140

Val Ile Thr Leu Ala Asp Trp Tyr His Thr Leu Ala Arg Gln Ile Val
145 150 155 160

Gly Val Ala Ile Ser Asp Thr Thr Leu Ile Asn Gly Leu Gly Arg Asn
165 170 175

Thr Asn Gly Pro Ala Asp Ala Ala Leu Ala Val Ile Asn Val Asp Ala

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            180                 185                 190

Gly Lys Arg Tyr Arg Phe Arg Leu Val Ser Ile Ser Cys Asp Pro Asn
        195                 200                 205

Trp Val Phe Ser Ile Asp Asn His Asp Phe Thr Val Ile Glu Val Asp
    210                 215                 220

Gly Val Asn Ser Gln Pro Leu Asn Val Asp Ser Val Gln Ile Phe Ala
225                 230                 235                 240

Gly Gln Arg Tyr Ser Phe
                245
```

<210> SEQ ID NO 13
<211> LENGTH: 2809
<212> TYPE: DNA
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 13

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gattctaata gaccaggcat accaagagat ctacaggttg acagaccatt cttctaggcg     60

gcatttatgc tgtagcgtca gaaattatct ctccatttgt atcccacagg tcctgtaata    120

acacggagac agtccaaact gggatgcctt ttttctcaac tatgggcgca catagtctgg    180

acgatggtat ataagacgat ggtatgagac ccatgaagtc agaacacttt tgctctctga    240

catttcatgg ttcacactct cgagatggga ttgaactcgg ctattacatc gcttgctatc    300

ttagctctgt cagtcggaag ctatgctgca attgggcccg tggccgacat acacattgtc    360

aacaaagacc ttgctccaga tggcgtacaa cgtccaaccg tgcttgccgg aggcactttt    420

cctgggacgt tgatcaccgg tcagaaagta agggatatta gtttgcgtca aagagccaac    480

caaaactaac cgtcccgtac tatagggtga caacttccag ctcaatgtca tcgatgatct    540

taccgacgat cggatgttga cgccaacttc cattgtgagc ctattattgt atgatttatc    600

cgaatagttt cgcagtctga tcattggatc tctatcgcta gcattggcac ggtttcttcc    660

agaagggaac cgcttgggcc gacggtcccg ccttcgtaac tcagtgccct ataatagcag    720

ataactcttt tctgtatgac ttcgacgtcc cagaccaagc tggtactttc tggtatcata    780

gtcatctatc cactcagtac tgtgacggtt tacgtggtgc cttcgttgtg tacgatccta    840

acgatcctca caaagaccta tacgatgttg atgacggtgg gttccaaata tttgttctgc    900

agacattgta ttgacggtgt tcattataat ttcagagagc accgtgatta cccttgcgga    960

ttggtaccat gttctcgccc agaccgttgt cggcgctgcg tgagtaacac atacacgcgc   1020

tccggcacac tgatactaat ttttttttat tgtagcactc ctgattctac cttgatcaac   1080

gggttaggcc gttcacagac cggacccgct gatgctgagc tggctgttat cagcgttgaa   1140

cataacaaac ggtatgtcat ctctacccag tatcttctct cctgctctaa ttcgctgttt   1200

caccatagat accgtttccg tttggtttcg atttcgtgcg accccaactt taccttctcc   1260

gttgatggtc ataatatgac tgtcatcgaa gtcgatggtg tcaacacacg acccctgacc   1320

gttgactcta ttcaaatctt cgccggacag aggtattcct ttgtcgtaag ttaatcgata   1380

tattctcctt attacccctg tgtaattgat gtcaatagct caatgctaac caacccgaag   1440

acaattactg gatccgtgct atgccaaaca tcggtagaaa tacaacaaca ctggacggaa   1500

agaatgccgc tatccttcga tacaagaatg cttctgtaga agagcccaag accgttgggg   1560

gccccgctca atccccgttg aatgaagcgg acctgcgtcc actcgtacct gctcctgtgg   1620

tatgtcttgt cgcgctgttc catcgctatt tcatattaac gttttgtttt tgtcaagcct   1680

ggaaacgctg ttccaggtgg cgcagacatc aatcacaggc ttaacttaac tttcgtacgt   1740
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acacctggtt gaaacattat atttccagtc taacctctct tgtagagtaa cggcctcttc 1800

agcatcaaca acgcctcctt cactaatcct tcggtccccg ccttattaca aattctgagc 1860

ggtgctcaga acgctcaaga tttacttcca acgggtagtt acattggcct tgaactaggc 1920

aaggttgtgg agctcgttat acctcctctg gcagttggag gaccgcaccc tttccatctt 1980

catggcgtaa gcataccaca ctcccgcagc cagaatgacg caaactaatc atgatatgca 2040

gcacaatttc tgggtcgtcc gtagtgcagg tagcgatgag tataactttg acgatgctat 2100

cctcagggac gtcgtragca ttggagcggg gactgatgaa gtcacaatcc gtttcgtggt 2160

atgtctcacc cctcgcattt tgagacgcaa gagctgatat attttaacat agaccgacaa 2220

tccgggcccg tggttcctcc attgccatat tgattggcat ttggaggcag gccttgccat 2280

cgtcttcgct gagggcatca atcagaccgc tgcagccaac ccaacacccc gtacgtgaca 2340

ctgagggttt ctttatagtg ctggattact gaatcgagat ttctccacag aagcatggga 2400

tgagctttgc cccaaatata acgggttgag tgcgagccag aaggtcaagc ctaagaaagg 2460

aactgctatt taaacgtggt cctagactac gggcatataa gtattcgggt agcgcgtgtg 2520

agcaatgttc cgatacacgt agattcatca ccggacacgc tgggacaatt tgtgtataat 2580

ggctagtaac gtatctgagt tctggtgtgt agttcaaaga gacagccctt cctgagacag 2640

cccttcctga gacagccctt cctgagacgt gacctccgta gtctgcacac gatactycta 2700

aatacgtatg gcaagatgac aaagaggagg atgtgagtta ctacgaacag aaatagtgcc 2760

cggcctcgga gagatgttct tgaatatggg actgggacca acatccgga 2809

<210> SEQ ID NO 14
<211> LENGTH: 526
<212> TYPE: PRT
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 14

Met Gly Leu Asn Ser Ala Ile Thr Ser Leu Ala Ile Leu Ala Leu Ser
1 5 10 15

Val Gly Ser Tyr Ala Ala Ile Gly Pro Val Ala Asp Ile His Ile Val
20 25 30

Asn Lys Asp Leu Ala Pro Asp Gly Val Gln Arg Pro Thr Val Leu Ala
35 40 45

Gly Gly Thr Phe Pro Gly Thr Leu Ile Thr Gly Gln Lys Gly Asp Asn
50 55 60

Phe Gln Leu Asn Val Ile Asp Asp Leu Thr Asp Asp Arg Met Leu Thr
65 70 75 80

Pro Thr Ser Ile His Trp His Gly Phe Phe Gln Lys Gly Thr Ala Trp
85 90 95

Ala Asp Gly Pro Ala Phe Val Thr Gln Cys Pro Ile Ile Ala Asp Asn
100 105 110

Ser Phe Leu Tyr Asp Phe Asp Val Pro Asp Gln Ala Gly Thr Phe Trp
115 120 125

Tyr His Ser His Leu Ser Thr Gln Tyr Cys Asp Gly Leu Arg Gly Ala
130 135 140

Phe Val Val Tyr Asp Pro Asn Asp Pro His Lys Asp Leu Tyr Asp Val
145 150 155 160

Asp Asp Gly Gly Thr Val Ile Thr Leu Ala Asp Trp Tyr His Val Leu
165 170 175

Ala Gln Thr Val Val Gly Ala Ala Thr Pro Asp Ser Thr Leu Ile Asn
180 185 190

-continued

```
Gly Leu Gly Arg Ser Gln Thr Gly Pro Ala Asp Ala Glu Leu Ala Val
        195                 200                 205

Ile Ser Val Glu His Asn Lys Arg Tyr Arg Phe Arg Leu Val Ser Ile
    210                 215                 220

Ser Cys Asp Pro Asn Phe Thr Phe Ser Val Asp Gly His Asn Met Thr
225                 230                 235                 240

Val Ile Glu Val Asp Gly Val Asn Thr Arg Pro Leu Thr Val Asp Ser
                245                 250                 255

Ile Gln Ile Phe Ala Gly Gln Arg Tyr Ser Phe Val Leu Asn Ala Asn
            260                 265                 270

Gln Pro Glu Asp Asn Tyr Trp Ile Arg Ala Met Pro Asn Ile Gly Arg
        275                 280                 285

Asn Thr Thr Thr Leu Asp Gly Lys Asn Ala Ala Ile Leu Arg Tyr Lys
    290                 295                 300

Asn Ala Ser Val Glu Glu Pro Lys Thr Val Gly Gly Pro Ala Gln Ser
305                 310                 315                 320

Pro Leu Asn Glu Ala Asp Leu Arg Pro Leu Val Pro Ala Pro Val Pro
                325                 330                 335

Gly Asn Ala Val Pro Gly Gly Ala Asp Ile Asn His Arg Leu Asn Leu
            340                 345                 350

Thr Phe Ser Asn Gly Leu Phe Ser Ile Asn Asn Ala Ser Phe Thr Asn
        355                 360                 365

Pro Ser Val Pro Ala Leu Leu Gln Ile Leu Ser Gly Ala Gln Asn Ala
    370                 375                 380

Gln Asp Leu Leu Pro Thr Gly Ser Tyr Ile Gly Leu Glu Leu Gly Lys
385                 390                 395                 400

Val Val Glu Leu Val Ile Pro Pro Leu Ala Val Gly Gly Pro His Pro
                405                 410                 415

Phe His Leu His Gly His Asn Phe Trp Val Val Arg Ser Ala Gly Ser
            420                 425                 430

Asp Glu Tyr Asn Phe Asp Asp Ala Ile Leu Arg Asp Val Val Ser Ile
        435                 440                 445

Gly Ala Gly Thr Asp Glu Val Thr Ile Arg Phe Val Thr Asp Asn Pro
    450                 455                 460

Gly Pro Trp Phe Leu His Cys His Ile Asp Trp His Leu Glu Ala Gly
465                 470                 475                 480

Leu Ala Ile Val Phe Ala Glu Gly Ile Asn Gln Thr Ala Ala Ala Asn
                485                 490                 495

Pro Thr Pro Gln Ala Trp Asp Glu Leu Cys Pro Lys Tyr Asn Gly Leu
            500                 505                 510

Ser Ala Ser Gln Lys Val Lys Pro Lys Lys Gly Thr Ala Ile
        515                 520                 525
```

<210> SEQ ID NO 15
<211> LENGTH: 2299
<212> TYPE: DNA
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 15

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gatctggacg atggtatata agacgatggt atgagaccca tgaagtctga acacttttgc     60

tctctgacat ttcatggttc atactctcga gatgggattg aactcggcta ttacatcgct    120

tgctatctta gctctgtcag tcggaagcta tgctgcaatt gggcccgtgg ccgacataca    180

cattgtcaac aaagaccttg ctccagatgg tgtacaacgt ccaaccgtgc tcgccggagg    240

cacttttcct gggacgttga tcaccggtca gaaagtaagg aatattagtt tgcgtcaaag    300
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agccaaccaa aattaaccgt cccgtcccat agggtgacaa cttccagctc aatgtcattg 360

atgatcttac cgacgatcgg atgttgacac caacttccat tgtgagccta ttattgtatg 420

atttatccgt atagtttctc agtctgatca ttggctctct atcgctagca ttggcacggt 480

ttcttccaga agggaaccgc ttgggccgac ggtcccgcct tcgtaactca gtgccctata 540

atagcagata actcttttct gtatgacttc gacgtccccg accaagctgg tactttctgg 600

tatcatagtc atctatccac tcagtactgt gacggtttac gtggtgcctt cgttgtgtac 660

gatcctaacg atcctcacaa agacctatac gatgttgatg acggtgggtt ccaaatactt 720

gaccaagaaa cattatattg atagtatcca ctctgatttt cagagagcac cgtgattacc 780

cttgcggatt ggtaccatgt tctcgcccag accgttgtcg gcgctgcgtg agtaacacat 840

acacgcgctc cggcacactg atactaattt tttattgtag cactcctgat tctaccttga 900

tcaacgggtt aggccgttca cagaccggac ccgctgatgc tgagctggct gttatcagcg 960

ttgaacataa caaacggtat gtcatctcta cccattatct tctctcctgc tttaattcgc 1020

tgtttcacca tagataccga ttccgtttgg tttcgatttc gtgcgacccc aactttacct 1080

tctccgttga tggtcataat atgactgtca tcgaagtcga cggtgtcaac acacgacccc 1140

tgaccgttga ctctattcaa atcttcgccg gacagaggta ttcctttgtc gtaagttaat 1200

cgatatattc tccctattac ccctgtgtaa ttgatgtcaa cagctcaatg ctaaccaacc 1260

cgacgacaat tactggatcc gtgctatgcc aaacatcggt agaaatacaa caacactgga 1320

cggaaagaat gccgctatcc ttcgatacaa gaatgcttct gtagaagagc ccaagaccgt 1380

tgggggcccc gctcaatccc cgttgaatga agcggacctg cgtccactcg tacctgctcc 1440

tgtggtatgt cttgtcgtgc tgttccatcg ctatttcata ttaacgtttt gtttttgtca 1500

agcctggaaa cgctgttcca ggtggcgcag acatcaatca caggcttaac ttaactttcg 1560

tacgtacacc tggttgaaac attatatttc cagtctaacc tcttgtagag taacggcctt 1620

ttcagcatca acaacgcctc cttcactaat ccttcggtcc ccgccttatt acaaattctg 1680

agcggtgctc agaacgctca agatttactt ccaacgggta gttacattgg ccttgaacta 1740

ggcaaggttg tggagctcgt tatacctcct ctggcagttg gaggaccgca ccctttccat 1800

cttcatggcg taagcatacc acactcccgc agccagaatg acgcaaacta atcatgatat 1860

gcagcacaat ttctgggtcg tccgtagtgc aggtagcgat gagtataact ttgacgatgc 1920

tatcctcagg gacgtcgtga gcattggagc ggggactgat gaagtcacaa tccgtttcgt 1980

ggtatgtctc acccctcgca ttttgagacg caagagctga tatattttaa catagaccga 2040

caatccgggc ccgtggttcc tccattgcca tattgattgg catttggagg caggccttgc 2100

catcgtcttc gctgagggca tcaatcagac cgctgcagcc aacccaacac cccgtacgtg 2160

acactgaggg tttctttata gtgctggatt actgaatcga gatttctcca cagaagcatg 2220

ggatgagctt tgccccaaat ataacgggtt gagtgcgagc cagaaggtca agcctaagaa 2280

aggaactgct atttaaacg 2299

<210> SEQ ID NO 16
<211> LENGTH: 526
<212> TYPE: PRT
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 16

Met Gly Leu Asn Ser Ala Ile Thr Ser Leu Ala Ile Leu Ala Leu Ser
1 5 10 15

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```
Val Gly Ser Tyr Ala Ala Ile Gly Pro Val Ala Asp Ile His Ile Val
            20                  25                  30

Asn Lys Asp Leu Ala Pro Asp Gly Val Gln Arg Pro Thr Val Leu Ala
        35                  40                  45

Gly Gly Thr Phe Pro Gly Thr Leu Ile Thr Gly Gln Lys Gly Asp Asn
    50                  55                  60

Phe Gln Leu Asn Val Ile Asp Asp Leu Thr Asp Asp Arg Met Leu Thr
65                  70                  75                  80

Pro Thr Ser Ile His Trp His Gly Phe Phe Gln Lys Gly Thr Ala Trp
                85                  90                  95

Ala Asp Gly Pro Ala Phe Val Thr Gln Cys Pro Ile Ile Ala Asp Asn
            100                 105                 110

Ser Phe Leu Tyr Asp Phe Asp Val Pro Asp Gln Ala Gly Thr Phe Trp
        115                 120                 125

Tyr His Ser His Leu Ser Thr Gln Tyr Cys Asp Gly Leu Arg Gly Ala
    130                 135                 140

Phe Val Val Tyr Asp Pro Asn Asp Pro His Lys Asp Leu Tyr Asp Val
145                 150                 155                 160

Asp Asp Gly Gly Thr Val Ile Thr Leu Ala Asp Trp Tyr His Val Leu
                165                 170                 175

Ala Gln Thr Val Val Gly Ala Ala Thr Pro Asp Ser Thr Leu Ile Asn
            180                 185                 190

Gly Leu Gly Arg Ser Gln Thr Gly Pro Ala Asp Ala Glu Leu Ala Val
        195                 200                 205

Ile Ser Val Glu His Asn Lys Arg Tyr Arg Phe Arg Leu Val Ser Ile
    210                 215                 220

Ser Cys Asp Pro Asn Phe Thr Phe Ser Val Asp Gly His Asn Met Thr
225                 230                 235                 240

Val Ile Glu Val Asp Gly Val Asn Thr Arg Pro Leu Thr Val Asp Ser
                245                 250                 255

Ile Gln Ile Phe Ala Gly Gln Arg Tyr Ser Phe Val Leu Asn Ala Asn
            260                 265                 270

Gln Pro Asp Asp Asn Tyr Trp Ile Arg Ala Met Pro Asn Ile Gly Arg
        275                 280                 285

Asn Thr Thr Thr Leu Asp Gly Lys Asn Ala Ala Ile Leu Arg Tyr Lys
    290                 295                 300

Asn Ala Ser Val Glu Glu Pro Lys Thr Val Gly Gly Pro Ala Gln Ser
305                 310                 315                 320

Pro Leu Asn Glu Ala Asp Leu Arg Pro Leu Val Pro Ala Pro Val Pro
                325                 330                 335

Gly Asn Ala Val Pro Gly Gly Ala Asp Ile Asn His Arg Leu Asn Leu
            340                 345                 350

Thr Phe Ser Asn Gly Leu Phe Ser Ile Asn Asn Ala Ser Phe Thr Asn
        355                 360                 365

Pro Ser Val Pro Ala Leu Leu Gln Ile Leu Ser Gly Ala Gln Asn Ala
    370                 375                 380

Gln Asp Leu Leu Pro Thr Gly Ser Tyr Ile Gly Leu Glu Leu Gly Lys
385                 390                 395                 400

Val Val Glu Leu Val Ile Pro Pro Leu Ala Val Gly Gly Pro His Pro
                405                 410                 415

Phe His Leu His Gly His Asn Phe Trp Val Val Arg Ser Ala Gly Ser
            420                 425                 430

Asp Glu Tyr Asn Phe Asp Asp Ala Ile Leu Arg Asp Val Val Ser Ile
        435                 440                 445
```

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```
Gly Ala Gly Thr Asp Glu Val Thr Ile Arg Phe Val Thr Asp Asn Pro
    450                 455                 460

Gly Pro Trp Phe Leu His Cys His Ile Asp Trp His Leu Glu Ala Gly
465                 470                 475                 480

Leu Ala Ile Val Phe Ala Glu Gly Ile Asn Gln Thr Ala Ala Ala Asn
                485                 490                 495

Pro Thr Pro Gln Ala Trp Asp Glu Leu Cys Pro Lys Tyr Asn Gly Leu
            500                 505                 510

Ser Ala Ser Gln Lys Val Lys Pro Lys Lys Gly Thr Ala Ile
        515                 520                 525
```

<210> SEQ ID NO 17
<211> LENGTH: 1163
<212> TYPE: DNA
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 17

```
tgcaatcgga ccggtggccg acctcaagat cgtaaaccga gacattgcac ctgacggttt     60

tattcgtccc gccgttctcg ctggagggtc gttccctggt cctctcatta cagggcagaa    120

agtacgttac gctatctcgg tgctttggct taattaaact atttgacttt gtgttctctt    180

aggggaacga gttcaaaatc aatgtagtca atcaactgac cgatggttct atgttaaaat    240

ccacctcaat cgtaagcaga atgagccctt tgcatctcgt tttattgtta atgcgcccac    300

tatagcattg gcatggattc ttccagaagg gaacaaactg ggcagacggt cctgcgttcg    360

tgaaccaatg tccaatcgcc acgaacaatt cgttcttgta tcagtttacc tcacaggaac    420

agccaggtga gtatgagatg gagttcatcc gagcatgaac tgatttattt ggaacctagg    480

cacattttgg taccatagtc atctttccac acaatactgc gatggtttgc gagggccact    540

cgtggtgtat gacccacaag acccgcatgc tgttctctac gacgtcgacg atggttcgta    600

cttcgcatat ccacgctcgc tttcatacaa tgtaaacttt gttcctccag aaagtacaat    660

catcacgctc gcggattggt atcatacctt ggctcggcaa gtgaaaggcc cagcgtaagg    720

cactttagtg tttcctcata gtccaagaaa ttctaacacg ccttcttcat cagggttcct    780

ggtacgacct tgatcaacgg gttggggcgt cacaacaatg gtcctctaga tgctgaacta    840

gcggtgatca gtgttcaagc cggcaaacgg caagttcaat tcacactttt cactctgtac    900

cttcttcctg acattctttt cttgtagtta ccgcttccgc ctgatttcaa tttcatgcga    960

tcccaactac gtattctcca ttgatggcca tgatatgact gtcatcgaag tggatagtgt   1020

taacagtcaa cctctcaagg tagattctat ccaaatattt gcaggtcaga gatattcgtt   1080

cgtggtgagt cagatcaggg catatccttt tgtcgatacg tcattgacca tataatgcta   1140

caagctgaat gccaaccaac cag                                           1163
```

<210> SEQ ID NO 18
<211> LENGTH: 262
<212> TYPE: PRT
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 18

```
Ala Ile Gly Pro Val Ala Asp Leu Lys Ile Val Asn Arg Asp Ile Ala
1               5                   10                  15

Pro Asp Gly Phe Ile Arg Pro Ala Val Leu Ala Gly Gly Ser Phe Pro
            20                  25                  30

Gly Pro Leu Ile Thr Gly Gln Lys Gly Asn Glu Phe Lys Ile Asn Val
        35                  40                  45
```

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```
Val Asn Gln Leu Thr Asp Gly Ser Met Leu Lys Ser Thr Ser Ile His
    50                  55                  60

Trp His Gly Phe Phe Gln Lys Gly Thr Asn Trp Ala Asp Gly Pro Ala
65                  70                  75                  80

Phe Val Asn Gln Cys Pro Ile Ala Thr Asn Asn Ser Phe Leu Tyr Gln
                85                  90                  95

Phe Thr Ser Gln Glu Gln Pro Gly Thr Phe Trp Tyr His Ser His Leu
            100                 105                 110

Ser Thr Gln Tyr Cys Asp Gly Leu Arg Gly Pro Leu Val Val Tyr Asp
        115                 120                 125

Pro Gln Asp Pro His Ala Val Leu Tyr Asp Val Asp Asp Glu Ser Thr
    130                 135                 140

Ile Ile Thr Leu Ala Asp Trp Tyr His Thr Leu Ala Arg Gln Val Lys
145                 150                 155                 160

Gly Pro Ala Val Pro Gly Thr Thr Leu Ile Asn Gly Leu Gly Arg His
                165                 170                 175

Asn Asn Gly Pro Leu Asp Ala Glu Leu Ala Val Ile Ser Val Gln Ala
            180                 185                 190

Gly Lys Arg Gln Val Gln Phe Thr Leu Phe Thr Leu Tyr Arg Phe Arg
        195                 200                 205

Leu Ile Ser Ile Ser Cys Asp Pro Asn Tyr Val Phe Ser Ile Asp Gly
    210                 215                 220

His Asp Met Thr Val Ile Glu Val Asp Ser Val Asn Ser Gln Pro Leu
225                 230                 235                 240

Lys Val Asp Ser Ile Gln Ile Phe Ala Gly Gln Arg Tyr Ser Phe Val
                245                 250                 255

Leu Asn Ala Asn Gln Pro
            260


<210> SEQ ID NO 19
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 19

Ala Ile Gly Pro Val Ala Asp Leu His Ile
1               5                   10


<210> SEQ ID NO 20
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 20

Met Leu Thr Pro Thr Ser Ile
1               5


<210> SEQ ID NO 21
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 21

Thr Val Gly Gly Pro Ala
1               5


<210> SEQ ID NO 22
<211> LENGTH: 10
<212> TYPE: PRT
```

-continued

<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 22

Tyr Ser Phe Val Leu Asn Ala Asn Gln Pro
1 5 10

<210> SEQ ID NO 23
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 23

gcaatcggac cngtngcaga 20

<210> SEQ ID NO 24
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 24

gcaatcggac cngtngctga 20

<210> SEQ ID NO 25
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 25

gcaatcggac cngtngcgga 20

<210> SEQ ID NO 26
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature

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<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 26

gcaatcggac cngtngccga 20

<210> SEQ ID NO 27
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (18)..(18)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 27

ggttgatttg cattnagnac 20

<210> SEQ ID NO 28
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (18)..(18)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 28

ggttgatttg cgttnagnac 20

<210> SEQ ID NO 29
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 29

ggacgtggcc ttgagcatac 20

<210> SEQ ID NO 30
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 30

tctgtcaagt cgtcaatcac 20

<210> SEQ ID NO 31
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature

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<222> LOCATION: (1)..(10)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 31

nnnnnnnnnn ggatcc 16

<210> SEQ ID NO 32
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 32

ttaccacgaa tcagaggacc 20

<210> SEQ ID NO 33
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 33

cctcacctgt attggcacag 20

<210> SEQ ID NO 34
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 34

ttggtatcat gcccttgctc 20

<210> SEQ ID NO 35
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 35

ttcgcaggtc aacgatattc 20

<210> SEQ ID NO 36
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 36

gttaggtggt tgaaggattg 20

<210> SEQ ID NO 37
<211> LENGTH: 1316
<212> TYPE: DNA
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 37

ttcgcaggtc aacgatattc gttcgtcgta agtctctttg aacgattact gcttctttgt 60

ccattctctg acctgtttaa acagctccat gccaaccgtc ctgaaaacaa ctattggatc 120

agggccaaac ctaatatcgg tacggatact accacagaca acggcatgaa ctctgccatt 180

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ctgcgataca acggcgcacc tgttgcggaa ccgcaaactg ttcaatctcc cagtctcacc 240

cctttgctcg aacagaacct tcgccctctc gtgtacactc ctgtggtatg tttcaaagcg 300

ttgtaatttg attgtggtca ttctaacgtt actgcgtttg catagcctgg aaatcctacg 360

cctggcggcg ccgatattgt ccatattctt gacttgagtt ttgtgcggag tcaacattcg 420

taaagatgag agtgtttcta atttcttcaa taataggatg ctggtcgctt cagtatcaac 480

ggtgcctcgt tccttgatcc taccgtcccc gttctcctgc aaattctcag cggcacgcag 540

aatgcacaag atctactccc tcctggaagt gtgattcctc tcgaattagg caaggtcgtc 600

gaattagtca tacctgcagg tgtcgtcggt ggacctcatc cgttccatct ccatggggta 660

cgtaacccga acttataaca gtcttggact tacccgctga caagtgtata gcataacttc 720

tgggtcgtgc gaagtgccgg aaccgaccag tacaacttta acgatgccat tctccgagac 780

gtcgtcagta taggaggaac cggggatcaa gtcaccattc gtttcgtggt atgtttcatt 840

cttgtggatg tatgtgctct aggatactaa ccggcttacg cgtatagacc gataaccccg 900

gaccgtggtt cctccattgc catatcgact ggcacttgga agcgggtctc gctatcgtat 960

ttgcagaggg aattgaaaat actgctgcgt ctaatccaac cccccgtacg tggtttccct 1020

cacatcgtgg atctaagcag cttactaaca tacatttgca gaggcttggg atgagctttg 1080

cccgaagtat aacgcgctca gcgcacaaaa gaaggttgca tctaagaaag gcactgccat 1140

ctaatttttg taacaaacaa ggagggtctc ttgtactttt attgggattt atttcttggg 1200

gtttgttgtt caacttgatt ctactatgtt tggaagtagc gatgacgaaa ggggctcgcg 1260

catttatata ctatctctct tggcatcacc tgcagctcaa tccttcaacc acctaa 1316

<210> SEQ ID NO 38
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(10)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 38

nnnnnnnnnn cgatcg 16

<210> SEQ ID NO 39
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 39

caatctatga ccgtagattc 20

<210> SEQ ID NO 40
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 40

accgtggttc ctccattgcc 20

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<210> SEQ ID NO 41
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 41

gactggcact tggaagcggg 20

<210> SEQ ID NO 42
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 42

ggaccaagct ggtactttc 19

<210> SEQ ID NO 43
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 43

cgtggtacca gtctgccagg g 21

<210> SEQ ID NO 44
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 44

ggcagcatca gtcacggtca g 21

<210> SEQ ID NO 45
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 45

caccagcatg agctcaaagc tac 23

<210> SEQ ID NO 46
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 46

caccgcgatg tctcttcttc gtag 24

<210> SEQ ID NO 47
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 47

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tgragrtgga asggatgwgg tcc 23

<210> SEQ ID NO 48
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 48

gtccctgtac tactccagat cc 22

<210> SEQ ID NO 49
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 49

ccagcaggaa gcgtgatcga ac 22

<210> SEQ ID NO 50
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 50

gtaatcatgt atcacctggg ctcaagg 27

<210> SEQ ID NO 51
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (18)..(18)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 51

acgaacgagt ancgttgncc 20

<210> SEQ ID NO 52
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 52

Gly Gln Arg Tyr Ser Phe Val
1 5

<210> SEQ ID NO 53
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (11)..(11)

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<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 53

ctggttggtt ngcattnag 19

<210> SEQ ID NO 54
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Cerrena unicolor

<400> SEQUENCE: 54

Leu Asn Ala Asn Gln Pro
1 5

<210> SEQ ID NO 55
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 55

cacacgaccc ctgaccgttg 20

<210> SEQ ID NO 56
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 56

tgaccggtga tcaacgtccc 20

<210> SEQ ID NO 57
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 57

ggcgcagaca tcaatcacag 20

<210> SEQ ID NO 58
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 58

tcttcagcat caacaacgcc 20

<210> SEQ ID NO 59
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 59

tccggcaagc acggttgg 18

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<210> SEQ ID NO 60
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 60

tcgtcttcgc tgagggcatc 20

<210> SEQ ID NO 61
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 61

cagaccgctg cagccaaccc 20

<210> SEQ ID NO 62
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 62

aacacggaga cagtccaaac 20

<210> SEQ ID NO 63
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 63

cacctctcga gatgggattg aac 23

<210> SEQ ID NO 64
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 64

cgtttaaata gcagttcctt tc 22

<210> SEQ ID NO 65
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 65

ggactagtgt cgccgtttac aaacgcgcaa tcggtccagt cactgacc 48

<210> SEQ ID NO 66
<211> LENGTH: 2159
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic PCR fragment

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<400> SEQUENCE: 66

actagtgtcg ccgtttacaa acgcgcaatc ggtccagtca ctgacctaca tatagtgaac 60

cagaatctcg acccagatgg tttcaaccgc cccactgtac tcgcaggtgg tactttcccc 120

ggtcctctga ttcgtggtaa caaggtacgc ttcataaccg ccctccgtag acgtaggctt 180

cggctgacat gaccatcatc tgtagggaga taactttaaa attaatgtga ttgacgactt 240

gacagagcac agtatgctca aggctacgtc catcgtaagt ccctgattaa cgtttcacct 300

ggtcatatcg ctcaacgtct cgaagcactg gcatgggttc ttccagaagg gaaccaactg 360

ggccgatggc cccgcctttg tcacccaatg tcctatcaca tcaggaaacg ccttcctgta 420

tgatttcaac gttccggacc aagctggtac tttctggtac cacagccatc tctctacaca 480

gtattgtgac ggtcttcgtg gtgcctttgt cgtctatgat cctaatgatc ccaacaagca 540

actctatgat gttgataacg gcaagttcct tgcatatttc atttctatca tatcctcacc 600

tgtattggca cagaaagcac cgtgattacc ttggctgatt ggtatcatgc ccttgctcag 660

actgtcactg gtgtcgcgtg agtgacaaat ggccctcaat tgttcacata ttttcctgat 720

tatcatatga tagagtatct gatgcaacgt tgatcaacgg attgggacgt tcggccaccg 780

gccccgcaaa tgcccctctg gcggtcatca gtgtcgagcg gaataagagg tcagttccat 840

aattatgatt atttcccgcg ttacttccta acaattattt ttgtatccct ccacagatat 900

cgtttccgat tggtttctat ttcttgcgac cctaacttta ttttctcaat tgaccaccac 960

ccaatgaccg taattgagat ggacggtgtt aatacccaat ctatgaccgt agattcgatc 1020

caaatattcg caggtcaacg atattcattt gtcgtaggtt attataaact gcccaccgat 1080

catctctcac gtaactgtta tagatgcaag ccaaccaacc agttggaaat tattggatcc 1140

gcgctaaacc taatgttggg aacacaactt tccttggagg cctgaactcc gctatattac 1200

gatatgtggg agcccctgac caagaaccga ccactgacca aacacccaac tctacaccgc 1260

tcgttgaggc gaacctacga cccctcgtct atactcctgt ggtatgttgt tctcgttaca 1320

tataccaaac ctaatatgaa gactgaacgg atctactagc cgggacagcc attccctggc 1380

ggtgctgata tcgtcaagaa cttagctttg ggtttcgtac gtgtatttca cttccctttt 1440

ggcagtaact gaggtggaat gtatatagaa tgccgggcgt ttcacaatca atggagcgtc 1500

cctcacacct cctacagtcc ctgtactact ccagatcctc agtggtactc acaatgcaca 1560

ggatcttctc ccagcaggaa gcgtgatcga acttgaacag aataaagttg tcgaaatcgt 1620

tttgcccgct gcgggcgccg ttggcggtcc tcatcctttt cacttacatg gtgtaagtat 1680

cagacgtcct catgcccata ttgctccgaa ccttacacac ctgatttcag cacaatttct 1740

gggtggttcg tagcgccggt caaaccacat acaatttcaa tgatgctcct atccgtgatg 1800

ttgtcagtat tggcggtgca aacgatcaag tcacgatccg atttgtggta tgtatctcgt 1860

gccttgcatt cattccacga gtaatgatcc ttacacttcg ggttctcaga ccgataaccc 1920

tggcccatgg ttccttcact gtcacattga ctggcatttg gaggctgggt tcgctgtagt 1980

ctttgcggag ggaatcaatg gtactgcagc tgctaatcca gtcccaggta agactctcgc 2040

tgctttgcgt aatatctatg aatttaaatc atatcaattt gcagcggctt ggaatcaatt 2100

gtgcccattg tatgatgcct tgagcccagg tgatacatga ttacaagggt gggcgcgcc 2159

<210> SEQ ID NO 67
<211> LENGTH: 1523
<212> TYPE: DNA
<213> ORGANISM: Artificial

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<220> FEATURE:
<223> OTHER INFORMATION: synthetic codon optimized laccase B

<400> SEQUENCE: 67

actagtgtcg ccgtttacaa acgcgcaatc ggtcccgtca ctgacctgca tattgtgaac 60

cagaatctcg accccgatgg tttcaaccgc cccactgtcc tcgcaggtgg tactttcccc 120

ggtcctctga ttcgtggtaa caagggagat aactttaaaa ttaatgtgat tgacgacttg 180

acagagcaca gcatgctcaa ggctacgtcc atccactggc atggcttctt ccagaaggga 240

accaactggg ccgatggccc cgcctttgtc acccaatgtc ctatcacatc aggaaacgcc 300

ttcctgtacg atttcaacgt tccggaccaa gctggtactt tctggtacca cagccatctc 360

tctacacagt actgtgacgg tcttcgtggt gcctttgtcg tctacgatcc taatgatccc 420

aacaagcaac tctacgatgt tgataacggc aacaccgtga ttaccttggc tgattggtac 480

catgcccttg ctcagactgt cactggtgtc gcagtctctg atgcaacgtt gatcaacgga 540

ttgggacgtt cggccaccgg ccccgcaaat gcccctctgg cggtcatcag cgtcgagcgc 600

aataagcgct atcgtttccg attggtttct atttcttgcg accctaactt tattttctca 660

attgaccacc accccatgac cgtcattgag atggacggtg ttaataccca atctatgacc 720

gtagattcga tccaaatctt cgcaggtcaa cgatactcat ttgtcatgca agccaaccaa 780

ccagttggaa attactggat ccgcgctaaa cctaatgttg gcaacacaac tttccttgga 840

ggcctgaact ccgctatctt gcgatacgtg ggagcccctg accaagaacc gaccactgac 900

caaacaccca actctacacc gctcgttgag gcgaacctgc gacccctcgt ctacactcct 960

gtgccgggac agccattccc tggcggtgct gatatcgtca agaacttggc tttgggtttc 1020

aatgccgggc gtttcacaat caatggagcg tccctcacac ctcctacagt ccctgtcctg 1080

ctccagatcc tcagcggtac tcacaatgca caggatcttc tcccggcagg aagcgtgatc 1140

gaacttgaac agaataaagt tgtcgaaatc gttttgcccg ctgcgggcgc cgttggcggt 1200

cctcatcctt ttcacttgca tggtcacaat ttctgggtgg ttcgtagcgc cggtcaaacc 1260

acatacaatt tcaatgatgc tcctatccgt gatgttgtca gcattggcgg tgcaaacgat 1320

caagtcacga tccgatttgt gaccgataac cctggcccat ggttccttca ctgtcacatt 1380

gactggcatt tggaggctgg attcgctgtc gtctttgcgg agggaatcaa tggtactgca 1440

gctgctaatc ccgtcccggc ggcttggaat caattgtgcc cgttgtacga tgccttgagc 1500

ccgggtgata catgaggcgc gcc 1523

<210> SEQ ID NO 68
<211> LENGTH: 47
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 68

ggactagtgt cgccgtttac aaacgcgcaa ttgggcccgt ggccgac 47

<210> SEQ ID NO 69
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 69

aaggcgcgcc ttaaatagca gttcctttct tag 33

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<210> SEQ ID NO 70
<211> LENGTH: 1550
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic codon optimized laccase D

<400> SEQUENCE: 70

actagtgtcg ccgtttacaa acgcgctatt ggaccagttg ctgatctgca catcgttaac 60

aaggatttgg ccccagacgg cgtccagcgc ccaactgttc tggccggtgg aacttttccg 120

ggcacgctga ttaccggtca aaagggcgac aacttccagc tgaacgtgat tgatgacctg 180

accgacgatc gcatgttgac ccctacttcg atccattggc atggtttctt ccagaaggga 240

accgcctggg ccgacggtcc ggctttcgtt acacagtgcc ctattatcgc agacaactcc 300

ttcctctacg atttcgacgt tcccgaccag gcgggcacct tctggtacca ctcacacttg 360

tctacacagt actgcgacgg tctgcgcggt gccttcgttg tttacgaccc caacgaccct 420

cacaaggacc tttatgatgt cgatgacggt ggcacagtta tcacattggc tgactggtat 480

cacgtcctcg ctcagaccgt tgtcggagct gctacacccg actctacgct gattaacggc 540

ttgggacgca gccagactgg ccccgccgac gctgagctgg ccgttatctc tgttgaacac 600

aacaagagat accgtttcag actcgtctcc atctcgtgcg atcccaactt cacttttagc 660

gtcgacggtc acaacatgac ggttatcgag gttgatggcg tgaatacccg ccctctcacc 720

gtcgattcca ttcaaatttt cgccggccag cgatactcct ttgtgctgaa tgccaatcag 780

cccgaggata actactggat ccgcgctatg cctaacatcg gacgaaacac cactaccctt 840

gatggcaaga atgccgctat cctgcgatac aagaacgcca gcgttgagga gcccaaaacc 900

gtcggaggac ccgcgcagag cccattgaac gaggccgacc tgcgacctct ggtgcccgct 960

cctgtccctg gcaacgcagt tcctggtggt gcggacatca accaccgcct gaacctgaca 1020

ttcagcaacg gcctcttctc tatcaataac gcatcattta caaaccccag cgtccctgcc 1080

ttgttgcaga ttctttccgg cgcacaaaac gctcaggatc tgcttcccac cggttcttat 1140

atcggcttgg agttgggcaa ggtcgttgaa ctcgtgatcc ctcccttggc cgttggtggc 1200

ccccatccat tccacttgca cggccacaac ttttgggtcg tccgaagcgc tggttctgac 1260

gagtataatt tcgacgatgc aattttgcgc gacgtggtca gcattggcgc gggaactgac 1320

gaggttacta tccgttttgt cactgataac ccaggccctt ggttcctcca ttgccacatc 1380

gactggcacc tcgaagccgg cctcgccatt gttttcgccg aaggcatcaa tcaaaccgca 1440

gccgccaacc cgactccaca ggcctgggac gaactctgcc ccaagtataa cggactctcc 1500

gcttcccaga aagtgaagcc caagaaggga acagccatct aaggcgcgcc 1550

<210> SEQ ID NO 71
<211> LENGTH: 1507
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic codon optimized laccase D

<400> SEQUENCE: 71

ggatcctgaa gctatcggtc cggttgcaga tttacacatc gtaaacaaag atcttgcacc 60

tgacggcgtt caacgtccaa ctgtacttgc tggtggaaca ttccctggta cacttattac 120

tggtcaaaaa ggtgacaact tccaattaaa cgtaattgac gatcttacag atgaccgtat 180

gcttacaccg acttcaattc actggcacgg tttctttcaa aaaggaacag catgggctga 240

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tggtcctgca ttcgttacac aatgtccaat cattgctgat aactctttcc tttacgattt 300

tgacgttcct gatcaagctg gtacattctg gtatcactca cacttatcca cacaatactg 360

cgatggactt cgcggagctt tcgtagttta cgacccaaac gatcctcata aagaccttta 420

cgatgtagat gatggtggaa cagttatcac attagctgat tggtaccatg tacttgctca 480

aacagttgta ggtgcagcta caccagattc aacacttatc aatggattag gacgttctca 540

aactggtcct gctgacgcag aacttgctgt aatctctgtt gaacataaca aacgttacag 600

attccgtctt gttagcattt cttgcgatcc aaacttcaca ttttcagttg acggacataa 660

catgacagtt atcgaagtag atggtgtaaa cacacgtcca cttactgtag actctatcca 720

aatcttcgca ggacaacgtt actcattcgt attaaacgca aatcaaccag aagataacta 780

ctggattcgt gcaatgccaa acatcggacg taacactaca actcttgacg gcaaaaacgc 840

agctattctt cgttacaaaa acgcttctgt tgaagaacct aaaacagttg gtggaccagc 900

acaatcacca cttaacgaag ctgacttacg tccactggtt ccagcacctg tacctggaaa 960

cgctgtacca ggaggtgctg atattaatca tagacttaac cttactttct ctaacggtct 1020

gttctcaatc aacaacgctt cattcacaaa tccttcagtt ccagcacttt tacaaattct 1080

tagcggtgca caaaatgctc aggatctttt accaactgga tcttacattg gtcttgaact 1140

gggtaaagta gttgaattag taattcctcc gcttgctgta ggtggaccac atcctttcca 1200

tcttcacggt cataacttct gggttgtacg ttctgctggt tcagatgaat acaacttcga 1260

tgacgcaatt cttcgtgatg ttgtatctat tggtgctgga acagatgaag taactattcg 1320

tttcgtaaca gataaccctg gtccttggtt cttacattgt catatcgatt ggcatcttga 1380

agctggactt gctattgttt tcgctgaagg aatcaatcaa acagctgcag ctaacccaac 1440

acctcaagca tgggacgaat tatgtccaaa atacaacgca ctttctccag gagatactta 1500

aaagctt 1507

<210> SEQ ID NO 72
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 72

gcagatctgc gatgtctctt cttcgtagct tgac 34

<210> SEQ ID NO 73
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 73

gaggtcacct ctagatcatg tatcacctgg gctcaaggca tc 42

<210> SEQ ID NO 74
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 74

ttgctagcaa cgtgatctcc aagcgtgcaa tcggtccagt cactgaccta c 51

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<210> SEQ ID NO 75
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 75

acgcagcctg aactagttgc gatcctctag ag 32

<210> SEQ ID NO 76
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic primer

<400> SEQUENCE: 76

ctctgatcaa ggtcatcagg tgtcgcccgg ggacagg 37

<210> SEQ ID NO 77
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: synthetic Kex2 site

<400> SEQUENCE: 77

Asn Val Ile Ser Lys Arg
1 5

The invention claimed is:

1. A recombinant laccase having the amino acid sequence of SEQ ID NO: 14 or having an amino acid sequence that is at least 95% identical to SEQ ID NO: 14.

2. The recombinant laccase of claim **1**, wherein the laccase is at least 98% identical to SEQ ID NO: 14.

* * * * *